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The sixty-first annual meeting of the Association of Official Agricultural Chemists was held at the Shoreham Hotel, Washington, D. C., October 20, 21, and 22, 1947.

The meeting was called to order by the President, J. O. Clarke, on the morning of October 20, at 10:00 o'clock.

OFFICERS, COMMITTEES, REFEREES, AND ASSOCIATE REFEREES OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS FOR THE YEAR ENDING NOVEMBER 1948

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G. H. Marsh, Director, Chemistry Division, Montgomery, Ala.

Vice-President

L. S. Walker, Agricultural Experiment Station, Burlington, Vt.

Secretary-Treasurer Emeritus

W. W. Skinner, Kensington, Md.

Secretary-Treasurer

Henry A. Lepper, U. S. Food and Drug Administration,
Washington 25, D. C.

Additional Members of the Executive Committee

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H. A. Halvorson, St. Paul, Minn.
W. B. White, Washington, D. C.
J. O. Clarke, Chicago, Ill.

PERMANENT COMMITTEES

Recommendations of Referees

(Figures in parentheses refer to year in which appointment expires.)

W. F. REINDOLLAR (Bureau of Chemistry, State Department of Health, Baltimore 18, Md.), *Chairman*

SUBCOMMITTEE A: G. E. Grattan (1948) (Department of Agriculture, 79 Sussex St. Ottawa, Can.), *Chairman;* H. A. HALVORSON (1950), and E. L. GRIFFIN (1952).

- Feeding stuffs
  - Mineral mixed feed (calcium and iodine)
  - Lactose in mixed feeds
  - Fat in fish meal
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SUBCOMMITTEE B: **HARRY J. FISHER** (1948), (Conn. Agricultural Experiment Station, New Haven, Conn.), *Chairman;* G. R. CLARK (1950), and F. H. Wiley (1952).

- Naval stores
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SUBCOMMITTEE C: JOSEPH CALLOWAY (1950), (Food and Drug Administration-Washington 25, D. C.), *Chairman;* P. A. CLIFFORD (1952), and A. H. ROBERTSON (1954).

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  - Quarternary ammonium compounds
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  - Dichloroacetic acid
  - Formaldehyde
  - Mold-inhibitors, propionates
  - Thiourea
  - Dulcin
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  - Volatile oil in spices
  - Sugar, ash, and pungent principles in mustards
  - Preparation of sample, and fat in mayonnaise and salad dressing

SUBCOMMITTEE D: C. S. FERGUSON (1948) (State Department Public Health, Boston, Mass.), *Chairman;* KENNETH L. MILSTEAD (1950), and J. Walter Sale (1952).

- Alcoholic beverages
  - Hops
  - Yeast (total and yeast solids)
  - Inorganic elements in beer
  - Color and turbidity in beer
  - Distilled spirits (obscuration test for proof and test for aging)
  - Chromatographic absorption of wines
  - Caramel in alcoholic beverages
  - Cordials and liqueurs
  - Fusel oil in distilled spirits (official method)
  - Fusel oil in distilled spirits (rapid method)
  - Carbon dioxide in beer
  - Methanol
- Cacao products
  - Lecithin
  - Malt solids
  - Pectic acid
  - Cacao ingredients
  - Lactose
  - Fat
- Cereal foods
  - Starch in raw and cooked cereals
  - Fat acidity in grain, flour, corn meal, and whole wheat flour
  - Sugar in bread and other cereal foods
  - Benzoic acid in flour
  - Carbon dioxide in self-rising flour
  - Milk solids and butterfat in bread
  - Proteolytic activity of flour
  - Soybean flour
  - Soybean flour in foods (immunological tests)
  - Phosphated flour
  - Baked products (moisture, ash, protein, fat, and crude fiber)
  - Moisture in self-rising flour, and in pancake, waffle, and doughnut flours
  - Bromates in flour
  - Phosphorus
  - Unsaponifiable matter and sterols in cereal foods containing eggs
- Baking powders and baking chemicals
  - Carbon dioxide
- Flavors and non-alcoholic beverages
  - Beta-ionone
  - Lemon oils and extracts
  - Organic solvents in flavors
  - Glycerol, vanillin, and coumarin in vanilla and imitation vanilla
  - Emulsion flavors
  - Maple flavor concentrates and imitations
  - Diacetyl
- Fruits and fruit products
  - Titration of acids (electrometric)
  - Fruit acids
  - Fruit and sugar in frozen fruit
  - Water-insoluble solids
- Sugars and sugar products
  - Unfermented reducing substances in molasses
  - Drying methods
  - Densimetric and refractometric methods
  - Honey (free acid and adulterants)
  - Confectionery
  - Reducing sugars
  - Corn sirup and corn sugar
  - Color and turbidity in sugar products
- Waters, brine, and salt
  - Boron in water
  - Fluorine in salt

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- LACTOSE IN MIXED FEEDS:
  - C. W. Sievert, American Dry Milk Institute, Chicago 1, Ill.
- FAT IN FISH MEAL:
  - Maurice E. Stansby, Fish and Wildlife Service, Seattle 2, Wash.
- ADULTERATION OF CONDENSED MILK PRODUCTS:
  - P. B. Curtis, Purdue University, Lafayette, Ind.
- CRUDE FAT OR ETHER EXTRACT:
  - H. H. Hoffman, Department of Agriculture, Tallahassee, Fla.
- ACTIVITY OF YEAST:
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* Subjects listed are only those for which appointments have been made. See page 1 for complete list of subjects. New appointments as made will be announced in the JOURNAL.

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Subcommittee C

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CATALASE IN FROZEN VEGETABLES:
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Referee: Sumner C. Rowe, Food and Drug Administration, Washington 25, D. C.

COFFEE AND TEA:
Referee: Mary E. Ruffe, Food and Drug Administration, Chicago 7, Ill.

COLORING MATTERS IN FOODS:
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SAMPLING, FAT, AND MOISTURE IN CHEESE:
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VOLATILE OIL IN SPICES:
Dan Unger, Food and Drug Administration, New York 14, N. Y.
SUGAR, ASH, AND PUNGENT PRINCIPLES IN MUSTARDS:
F. M. Garfield, Food and Drug Administration, St. Louis 1, Mo.
SAMPLING, AND FAT IN MAYONNAISE AND SALAD DRESSING:
Sam D. Fine, Food and Drug Administration, Cincinnati 2, Ohio

Subcommittee D

ALCOHOLIC BEVERAGES:
Referee: J. Walter Sale, Food and Drug Administration, Washington 25, D. C.
YEAST (TOTAL AND YEAST SOLIDS):
Robert I. Tenney, Wahl-Henius Inst., 64 E. Lake St., Chicago, Ill.

INORGANIC ELEMENTS IN BEER:
Gordon H. Bendix, Continental Can Company, Inc., Chicago, Ill.
COLOR AND TURBIDITY IN BEER:
B. H. Nissen, Anheuser-Busch, Inc., St. Louis, Mo.
DISTILLED SPIRITS (OBSCURATION METHOD FOR PROOF AND TEST FOR AGING):
G. F. Beyer, Bureau of Internal Revenue, Washington 25, D. C.
CHROMATOGRAPHIC ABSORPTION OF WINES:
Peter Valaer, Bur. Internal Revenue, Washington 25, D. C.
CARAMEL IN ALCOHOLIC BEVERAGES:
Peter Valaer
CORDIALS AND LIQUEURS:
John B. Wilson, Food and Drug Administration, Washington 25, D. C.
FUSEL OIL IN DISTILLED SPIRITS (OFFICIAL METHOD):
G. F. Beyer
CARBON DIOXIDE IN BEER:
Irwin Stone, Wallerstein Laboratories, New York 16, N. Y.
METHANOL:
M. A. Amerine

CACAO PRODUCTS:
Referee: W. O. Winkler, Food and Drug Administration, Washington 25, D. C.
LECITHIN:
J. H. Bornmann, Food and Drug Administration, Chicago 7, Ill.
MALT SOLIDS:
E. W. Meyers, Hershey Chocolate Company, Hershey, Pa.
PECTIC ACID:
W. O. Winkler
CACAO INGREDIENTS:
W. O. Winkler
LACTOSE:
Frank V. Kenney, Jr., Walter Baker Co., Dorchester 24, Mass.
FAT:
Carl Stone, Food and Drug Administration, Cincinnati, Ohio

CEREAL FOODS:
Referee: V. E. Munsey, Food and Drug Administration, Washington 25, D. C.
STARCH IN RAW AND COOKED CEREALS:
W. H. FETZER, Clinton Industries, Inc., Clinton, Iowa.
FAT ACIDITY IN GRAIN, FLOUR, CORN MEAL, AND WHOLE WHEAT FLOUR
Lawrence Zeleny, Agricultural Research Center, Beltsville, Md.
BENZOIC ACID IN FLOUR:
V. E. Munsey
CARBON DIOXIDE IN SELF-RISING FLOUR:
R. A. Barackman, Victor Chemical Works, Chicago Heights, Ill.
MILK SOLIDS AND BUTTERFAT IN BREAD:
V. E. Munsey
PROTEOLYTIC ACTIVITY OF FLOUR:
Byron S. Miller, Federal Hard Wheat Quality Laboratory, Manhattan, Kans.
SOYBEAN FLOUR:
W. L. Taylor, General Mills, Inc., Minneapolis, Minn.

PHOSPHATED FLOUR:
Frank H. Collins, Food and Drug Administration, Cincinnati 2, Ohio
BAKED PRODUCTS (MOISTURE, ASH, PROTEIN, FAT, AND CRUDE FIBER):
N. H. Walker
BROMATES IN FLOUR:
W. F. Geddes, University of Minnesota, University Farm, St. Paul, Minn.
PHOSPHORUS:
V. E. Munsey
UNSAPONIFIABLE MATTER AND STEROLS IN CEREAL FOODS CONTAINING EGGS:
L. W. Ferris, Food and Drug Administration, Buffalo, N. Y.

BAKING POWDERS AND BAKING CHEMICALS:
Referee: V. E. Munsey, Food and Drug Administration, Washington 25, D. C.
CARBON DIOXIDE:
John E. Tatar, Standard Brands, Inc., 1015 Independence Blvd., Chicago, Ill.

FLAVORS AND NON-ALCOHOLIC BEVERAGES:
Referee: John B. Wilson, Food and Drug Administration, Washington 25, D. C.
BETA-IONONE:
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LEMON OILS AND EXTRACTS:
John B. Wilson
ORGANIC SOLVENTS IN FLAVORS:
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EMULSION FLAVORS:
John B. Wilson
MAPLE FLAVOR CONCENTRATES AND IMITATIONS:
J. H. Bornmann, Food and Drug Administration, Chicago 7, Ill.
DIACETYL:
John B. Wilson

FRUITS AND FRUIT PRODUCTS:
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TITRATION OF ACIDS:
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FRUIT ACIDS:
Fred Hillig, Food and Drug Administration, Washington 25, D. C.
FRUIT AND SUGAR IN FROZEN FRUIT:
R. A. Osborn
WATER-INSOLUBLE SOLIDS:
R. A. Osborn

SUGARS AND SUGAR PRODUCTS
Referee: C. F. Snyder, National Bureau of Standards, Washington 25, D. C.
UNFERMENTED REDUCING SUBSTANCES IN MOLASSES:
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DRYING METHODS:
Lester D. Hammond, National Bureau of Standards, Washington 25, D. C.
DENSIMETRIC AND REFRACTOMETRIC METHODS:
C. F. Snyder
HONEY (FREE ACID AND COMMERCIAL SYRUP ADULTERANTS):
George P. Walton, Production and Marketing Administration, Beverage and Miscellaneous Foods Division, Washington 25, D. C.

CONFECTIONERY:
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REDUCING SUGARS:
Emma J. McDonald, National Bureau of Standards, Washington 25, D. C.
CORN SIRUP AND CORN SUGAR:
G. T. Peckham, Jr., Clinton Company, Clinton, Iowa
COLOR AND TURBIDITY IN SUGAR PRODUCTS:
J. F. Brewster, National Bureau of Standards, Washington 25, D. C.

WATERS, BRINE, AND SALT:
Referee: Anna E. Mix, Food and Drug Administration, Washington 25, D. C.
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Anna E. Mix
FLUORINE IN SALT:
Anna E. Mix

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PRESIDENT'S ADDRESS*

THE DETECTION AND ESTIMATION OF FILTH AND DECOMPOSITION IN FOODS

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Consumers have always wanted clean food. Congress, recognizing the need for consumer protection, enacted the Food and Drugs Act of June 30, 1906, which defines a food as adulterated "if it consists in whole or in part of a filthy, decomposed, or putrid animal or vegetable substance. . . ." Similar definitions are found in many State laws and city ordinances. While the 1906 Federal law used the words "Filthy, decomposed, or putrid," other laws employed such terms as "tainted, rotten, corrupted, offensive, unclean, etc."

The Federal Food, Drug, and Cosmetic Act of June 25, 1938, perpetuates the definition of adulteration in slightly different and more effective language, ". . .if it consists in whole or in part of any filthy, putrid, or decomposed substance. . . ." The 1938 Act went one step further in declaring as adulterated, food which has been ". . .prepared, packed, or held under insanitary conditions whereby it may have become contaminated with filth." Thus, the 1938 law not only banned food which is filthy, putrid, or decomposed, but also attempted to correct conditions which may cause food to become filthy or decomposed through insanitary conditions.

Many of the provisions in the 1938 law resulted from changes as the bill passed through the Congress, and there were many bitter controversies on some proposals. It is interesting to note that the provisions dealing with filth, decomposition, and sanitation remained the same from the time the first version was introduced in 1933 until the final passage of the Act in 1938. This demonstrates a practically unanimous recognition by the Congress and the public of the need for sanitation in the production of our nation's food supply.

Dr. E. O. Jordan, in discussing food sanitation in 1931, had this to say—"In addition to the arguments for cleanliness in food handling based on the avoidance of specific food contamination, the aesthetic reasons for maintaining the cleanliness of food must not be lost sight of. Common decency requires that substances repugnant to the average consumer be kept out of any food. Smyrna figs, about which there has been much hue and cry, are certainly more appetizing if known to have been packed under cleanly conditions. Cow dung from healthy cattle may not actually cause disease or any sort of intestinal disturbance but that is no reason for allowing any of it to be mixed with milk. Even if it is practicable to manipulate unsound food in such a way that it is not injurious to health and its original condition is completely disguised, no consumer should be exposed to the unpleasantness of eating food that he would not have chosen had he known its history. The food supplied to the modern civilized human being should, in all fairness, be so safeguarded that the consumer is not beset by lurid imaginings about its possible wanderings through back alleys and pigsties. The clean and appetizing preparation and transportation of food is a worthy end in itself.'' (Food Poisoning and Food-Borne Infection, by Edwin Oakes Jordan, Univ. of Chicago Press, April, 1931).

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Some of our laws set numerical limits of bacterical counts and other indices of filth or decomposition to guide enforcement officials in carrying out the mandates of the law. The Federal Import Milk Act, for instance, not only requires certain farm inspections but sets definite limits of bacteria counts. The more general terms used in many laws, such as filthy, putrid, decomposed, etc., require the enforcement officer to establish means of determining when food is filthy, putrid, or decomposed. This presents a problem of no mean proportions to the food chemist and other scientific worker to develop methods for the detection of filth and decomposition in foods and to acquire the extensive data necessary to interpret the methods.

While the terms filthy, putrid, and decomposed have been interpreted by many trial courts, only a few interpretations of these terms are to be found in decisions of the higher courts. Presumably this is true because the terms of the law are so plain that there has been little occasion for the higher courts to speak on the subject. The courts have almost unanimously held that the terms filthy, putrid, and decomposed as used in the statute, should be given their common and usual meaning. It does not follow, that food, in order to be classed as filthy, putrid, or decomposed, must have obnoxious organoleptic characteristics to the ordinary consumer. Several court decisions clearly support the use of microscopic methods for the detection of these forms of adulteration. The Courts have also made it clear that contact with filthy substances is sufficient to classify a food as filthy.

The A.O.A.C. has recognized its obligation to develop and study methods to implement the enforcement of sanitary requirements of food laws. This is evidenced by reports of the Association which have for many years included papers on chemical, microscopic, and biological methods designed to detect and estimate filth and decomposition in food.

In their grosser forms, filth and decomposition are easily detected by taste, smell, and vision. Organoleptic methods will frequently reveal decomposition in food before it is processed, mixed, spiced, etc. but such tests, in many types of food, usually fail after the product has been manipulated, making it necessary to apply tests of a more scientific nature. For example, sour milk is easily detected by taste and odor, but after it has been neutralized and dried it requires a careful chemical examination to determine the true condition of the product. Bread may be made from flour contaminated by weevils and rodents. While the condition of the flour is often obvious before it is sifted and baked into bread, rodent hairs, insect and larva parts and excreta in the bread can only be detected by a careful laboratory examination. Thus the scientific worker is faced with the problem of finding in finished foods evidence of their true condition not obvious by the ordinary senses of sight, taste, or smell.

A number of methods of approach have been employed successfully in the laboratory to determine decomposition. These methods may be classified broadly as follows:

(1) The estimation and identification of various types of microorganisms that develop in food as decomposition progresses (bacteria counts in milk, mold counts on tomato products, and butter, etc.).

(2) The determination and identification of characteristic chemical products of decomposition (indole and volatile fatty acids in seafood, lactic acid in sour milk, etc.).

(3) The disappearances, or marked decrease, of a normal ingredient of the food (sugar in frozen eggs).

The general method of establishing the presence of filth in food has been the separation of insoluble particles and a microscopic study to determine the identity and estimate the amount. In a few non-processed foods the presence of certain bacteria is indicative of contamination of the food with filth. For example, the finding of certain strains of *E. coli* in crabmeat shows that the crabmeat has been contaminated with fecal material.

While I have not attempted a comprehensive study of all the literature on filth and decomposition, I would like to trace the development of a few lines of work which are milestones of progress in dealing with this important subject.

When the Food and Drugs Act of 1906 was passed, tomato puree, catsup, and related foods were not unfrequently made of rotten tomatoes, artifically colored and preserved with chemicals to look like wholesome food. These comminuted products lent themselves readily to the use of decomposed and otherwise unfit raw materials. B. J. Howard, head of the Microanalytical Division of the Bureau of Chemistry, undertook to develop a scientific method for the detection of decomposition in this class of products. Howard developed what has since become known as the Howard Mold Count. The Howard method was one of the first scientific procedures generally used to detect decomposition in food. Since its introduction it has received world-wide recognition not only by food law enforcement officials but also by the food producing industries.

The Howard Mold Count Method was originally published in 1911 in Bureau of Chemistry Circular 68. It first appeared as an A.O.A.C. method in the 1920 edition of the Methods of Analysis. In the beginning it was applied only to comminuted tomato products. In the latest edition of the Methods of Analysis, we find the Howard Mold Count in modified form, applied not only to comminuted tomato products but also to six other classes of foods, including such dissimilar products as butter and infant foods. While mold is a normal ingredient in a few foods, such as Roquefort and Blue cheese, it is not found in most foods unless they have undergone decomposition. Since the mold count technique measures the amount of dead mold, it can be applied to foods which have been processed by heat, and not only shows that decomposition is present but tells us something about the amount.

J. D. Wildman studied the application of the Howard method to butter and other dairy products. Working with R. L. Vandaveer, Wildman published a very comprehensive study on mold in commercial butter which showed a direct relationship between decomposition in the cream and the mold count of the butter churned therefrom.

Let us now consider methods for the detection and estimation of filth and extraneous matter in food. Since all extraneous matter is not filthy, the usual purpose in examining food for extraneous matter is to determine the amount and kind of filth it may contain. The earliest recorded work in this field was on fluid milk fifty or more years ago, where sediment testing was studied in Europe and this country. The sediment test is intended primarily to reveal contamination of milk with cow manure and other filthy substances. Early workers concerned themselves only with the insoluble portion of the cow manure and other visible elements of filth, and very little progress has been made over the years in the development of methods for estimating the soluble portion of cow manure in milk.

Although detection of the insoluble contaminants in milk is now carried out by a comparatively simple procedure, it is amazing to learn that some of the early workers attempted to make the problem most complex. The simple and obvious method requires only the filtering of the milk through a suitable pad or paper and an estimate of the amount of sediment visually by comparison with pads containing known amounts of sediment, as is standard practice today. The earlier workers, however, first tried to estimate the amount of visible sediment which settled to the bottom of a container on permitting the milk to stand for a definite period of time. This method was refined by applying centrifugal force. Other workers allowed the milk to stand, decanting part off the top and refilling with water, and repeating these operations until the sediment was free of milk. The sediment was then filtered and weighed. Some of the methods in use in the early days took as long as three days to complete a determination!

Even the earlier workers recognized the desirability of identifying the components of the sediment from milk in order to determine its character, as well as the amount. At the present time many regulatory agencies make microscopic examination of sediment pads.

In the early thirties B. J. Howard and his co-workers undertook the development of a comprehensive set of methods for the detection and estimation of filth in many different types of food. The work was later continued by Henry Welch after Howard's retirement. In his report to the Association as referee on filth methods Welch stated: "The methods which utilize a variety of procedures are, however based upon one or more of three principles:

(1) Solubility of the food or drug and insolubility of the extraneous material.

(2) The preferential wetting of insects, insect parts, rodent hairs, or other extraneous matter by oily liquids whereby they acquire a lighter specific gravity, which allows their separation by flotation methods; and

(3) Selective sedimentation in water or heavier-than-water liquids."

While these fundamental principles are simple, their application can become very complex in many products.

A very significant publication with respect to filth in food appeared in 1944 under the title "Microanalysis of Food and Drug Products." This work, which was prepared under Welch's direction by members of the then Microanalytical Division of the Food and Drug Administration, consists largely of background material on the general subject of filth in foods and drugs and information of value to assist workers in isolating and identifying elements of filth. Included are some of the basic filth methods.

For the first time, the sixth edition of the *Methods of Analysis* contains a chapter concerned entirely with the detection and estimation of extraneous materials in foods and drugs. This chapter of 36 pages presents methods for the determination of extraneous materials in many foods, including canned citrus juices, tea, cocoa, dairy products, nuts, cereals, and others.

The application of the methods to a wide variety of foods tests the skill of the careful worker. We have learned through experience that most of the A.O.A.C. methods yield the best results when followed in the minutest detail. However, since the filth methods do not deal with all types of food, and frequently individual samples present unusual difficulties, the chemist is constantly called upon to introduce variations of the methods of separation with samples having unusual characteristics. The chemist's aim should be to separate the filth as completely as possible so that it can be readily identified and estimated.

Quite probably the work done to date on microscopic methods has been of more value to food officials and to industry than the work on purely chemical methods for detecting filth and decomposition. However, considerable progress is being made in this field. Of particular value is the work of Fred Hillig on the determination of lactic acid and volatile fatty acids as indices of decomposition in dairy products, eggs, fish, etc. In connection with the work on volatile acids in eggs, particularly as applied to dried eggs, we should mention the paper of Lepper, Bartram, and Hillig which brought to bear bacteriological and organoleptic tests as well as chemical ones.

Patterson dealt very comprehensively with the broad aspects of decomposition in his 1944 report as general referee on decomposition. Indole has been studied as an index of decomposition in shell fish and reports have been published on canned oysters by King, Flynn, and Gowanlock, on canned clams and oysters by Beecham, and on shrimp by Duggan and Strasburger. Associate Referee Harris will report at this meeting on galacturonic acid as an index of decomposition (rot) in apple products. It is of interest to note that indole was studied a number of years ago as an index of decomposition in shrimp and at that time it was concluded that indole was not of value. Subsequent to the original investigation, more precise methods of analysis were developed. Using the more adequate methods, it was found that the formation of indole was characteristic of certain types of decomposition in shrimp.

Recently Cook and Steers studied the possible use of the enzymes trypsin and alkaline phosphatase as indicative of rodent and insect excreta in certain types of uncooked foods. Results appear to be promising and the study is being continued.

R. E. Duggan has studied uric acid in frozen eggs and showed that it is valuable in revealing the use of eggs contaminated with manure from dirty shells.

At the time of my own introduction to a food laboratory nearly 35 years ago there were presented many purely chemical and physical problems, such as the determination of protein, fat, fibre, ash, phosphoric acid, benzoic acid, and the host of other procedures required to detect the comparatively crude types of sophistication then employed. Many of you had similar experiences and many of you, as I did, had a wonderful, although sometimes disheartening, time with glycerol in vinegar. Our modern day food chemists are frequently first introduced to the microscope to learn to identify rodent hairs, insect parts, and the other significant elements of filth. They learn how to separate these tiny particles from the food material itself to identify them, and to evaluate their significance. They learn about mold counts and rot counts, and what they mean in terms of filthy and decomposed food. They learn about indole and other chemical indices of decomposition. They learn essentially to establish by laboratory examination the condition of sanitation under which a given lot of food was produced. The modern chemist must also, of course, learn the things that the "old timers" did. All in all, the regulatory food chemist of today is in a field embracing a wide variety of interests, a field to challenge his ingenuity and give him an opportunity for outstanding public service.

In the development of evidence of filth or decomposition in food products, the valuable information which can be obtained by means of factory inspection should not be overlooked. Frequently, a thorough factory inspection is the only means available at the present time for determining the true condition of many foods. This is especially true in those industries where filtration, sifting, and other manufacturing procedures successfully remove the evidence of filth or decomposition without removing the existence of filth or decomposition.

While the A.O.A.C. has never attempted to go beyond the collection of a sample in its formal methods, I wonder if we should not consider a study of factory inspection methods as a proper function of the Association. Certainly every food official has a responsibility to establish evidence of violation of the law. Chemists and other scientific workers have provided enforcement officials with most of the evidence in the past frequently using methods of the A.O.A.C. Some of the A.O.A.C. methods, for example, sediment testing of milk, are routinely used by inspectors in the field. Why should we not go one step further and provide the inspector, who is usually a scientifically trained man, with methods of inspection which would have the sanction of the A.O.A.C. and the respect and authority ascribed to the A.O.A.C. by the courts.

While I do not have in mind, at least in the beginning, the general subject of factory investigations, I do have in mind the establishment of a factory inspection technique for a few classes of factories which operate under the same general procedure and principles. For example, a factory inspection technique suitable for primary cheese factories, another to creameries, and another to packers of frozen strawberries. It should be borne in mind that any method which receives the full sanction of the A.O.A.C. must be subjected to collaborative study. I believe this fundamental is so precious that no method should ever be adopted as official until it has been studied collaboratively and found to be satisfactory when used by any competent worker. It is difficult to carry out collaborative work on many of the laboratory methods currently under study by the Association, since perishable foods are involved which are difficult to preserve and distribute for study to a number of laboratories. The problem of collaboration has been successfully overcome in many difficult fields and it need not present an insurmountable difficulty in dealing with factory inspection methods. One solution might be the independent inspection of the same factory at the same time using the same method by different workers and a comparison of their reports for consistency.

That the Association recognizes the importance of further work on filth and decomposition is evidenced by the current activity of two Referees; one for filth and the other for decomposition, and of six Associate Referees on filth and four on decomposition. Many problems in this field, as in all other fields of science, are pressing for solution. I would like to mention just a few broad fields where methods are badly needed.

Probably the most obnoxious contaminant of food comes from the unclean habits of human beings in handling food. The estimation of *E. coli* in some types of unprocessed food, for example in crabmeat, furnishes evidence of contamination with fecal material. In certain types of unprocessed dairy products, shelled nuts, etc., the presence of *E. coli* furnishes presumptive evidence of contamination with excreta of human or animal origin. Badly needed are methods for the detection of traces of human excreta in processed food. The amount likely to be present, originating as it does from filthy personal habits, will be minute, and this problem is thus one to challenge the imagination of chemists, microbiologists, and other workers in this field.

Methods for the detection of soluble filth should be developed without delay. Occasionally food manufacturers, instead of attempting to bring about fundamental corrections in sanitary practices, will attempt to meet the problem by filtering or sifting out insoluble filth in the hope that the filthy nature of the product will be obscured. (As expressed by Judge Hutcheson of the Fifth United States Circuit Court ". . . it was certainly not intended by Congress to leave packing stock butter manufacturers completely free to use in making their completed product any kind of filthy and putrid material they chose to use, in the faith, the substance of things hoped for, the evidence of things not seen, that, in the homely phrase, it will all come out in the wash . . . .") Factory inspection evidence as to the presence of soluble filth is difficult and expensive to obtain. The A.O.A.C. must provide methods whereby such hidden filth can be detected.

As a food decays, many chemical substances are formed whose determination should give the chemist a measure of decomposition. Possibly some substance or class of substances may be characteristic of protein decomposition, another of fat, and still another of carbohydrates. Unfortunately we are forced to deal with many types of decomposition producing a variety of different classes of chemical substances in food. If a limited number of chemical substances could be found to be characteristic of decomposition in the various types of food, the problem would be simplified. For example, a method for predominantly protein foods and another for predominantly fatty foods would be much more desirable than a method for fish and another for shrimp.

After a substance is found to be characteristic of decomposition in a food product, extensive studies are required to interpret the results on examination of specific lots of food. Ideally, the most valuable index is a substance which is completely absent in sound food but plentiful in decomposed food. Unfortunately, many indices now used are present in minute amounts when no decomposition has occurred, and only when larger amounts are found can we establish decomposition as a fact. We hope and expect that work in this field will reveal many indices of the first type.

Methods for the determination of filth and decomposition should be as simple as possible and, to be of most value, should be applicable to field use.

The Howard Mold Count Method and the sediment test methods on milk have been most valuable because they lend themselves to field use. Although it would be difficult to use many of the present methods in the field it is not too much to expect that some of them may later be modified and made available for field use. A field method for lactic acid (not titratable acidity) in dairy products is badly needed.

The type of research needed in the field of filth and decomposition is very time consuming, extensive, and as a rule, exceedingly complex. I have every confidence that the many able young men and women now engaged in this work are capable of giving us the answers to these numerous and difficult problems. These young workers are doing outstanding research, and bringing to bear intense interest and original thinking. As I study the reports of these real scientists, I feel humble in the face of the progress which they are making.

ORDER OF PUBLICATION

The reports of the committees presented on the last day of the annual meeting are given at the beginning of the proceedings, not in their chronological order. This arrangement will assist the referees, associate referees, and collaborators in planning and developing their year's work. The remainder of the proceedings will follow in their usual order.

Third Day

WEDNESDAY—AFTERNOON SESSION

REPORT OF THE EDITORIAL BOARD

By H. A. Lepper, *Chairman*

At our last meeting progress in the sale of the sixth edition of *Methods of Analysis* was reported as being the disposition, during the first year, of over one-half of the number printed. This year we sold all but 650 copies of the remaining 4,000.

Advantage has been taken by over 6,000 purchasers of the Book of the offer of the Association to furnish reprints of the report on changes in methods adopted at meetings subsequent to the publication of the book. This, together with the favorable comment on this service shows it has fulfilled a definite need. Reprints of changes made each year until publication of the seventh edition will be sent to those who have asked to be placed on the mailing list.

As with previous editions, the reviews devoted to the book in the scientific journals of the world have been most favorable. A few complaints have been directed to the decimal system of numbering the sections of the chapters on the assumption that with decimal numbers, for example, 24.41 should follow 24.4. With the use of the book, however, this possible confusion is soon overcome, and with the next printing the book can be avoided by the use of a colon or hyphen in place of the decimal point.

This year there has been no need for a committee on revision of methods, so we are to have only the report by Dr. White for the Committee on the Journal. The report of the Treasurer shows that the Journal has continued to be self-supporting.

Approved.

REPORT OF THE EDITORIAL COMMITTEE OF "THE JOURNAL"

W. B. White, *Editor and Chairman*

Despite annoying delays caused by the paper shortage and production difficulties at the printing plant, the *Journal* has shown gratifying progress during the year. Total pages will be 706 against 448 pages for Volume 29. There will be 38 excellent contributed papers which is 10 more than the number in Volume 29. More and more, contributors are finding that the *Journal* is an excellent medium from many standpoints: wide circulation, discriminating readers, speed of publication, and the high scientific caliber of our reviewers, to name only a few.

In the matter of our wide circulation, it may be of interest to say that all 48 States, the District of Columbia, Alaska, Puerto Rico, Hawaii, Panama, and the Philippines are represented. Beside Canada, England, Scotland, and Ireland, there are subscribers in 8 other British lands "overseas." Central and South America and the West Indian republics number 11 countries on our list, Europe 14 (including Iceland), Asia 4, and Africa 1 (Egypt). Subscribers now number nearly 1800, compared with about 1500 for Volume 29.

We have instituted the practice of promptly acknowledging receipt of manuscripts, with no commitments as to their acceptability. The "Notice to Contributors," which appeared in Volume 28, page 35 (Feb. 1945), is being enclosed with the acknowledgement. It will be mailed to others on request.

In closing, the Chairman wishes, on behalf of the Committee, to thank our reviewers for their conscientious and able reviews, and our contributors for the patience and unfailing courtesy they have shown toward editorial suggestions.

Approved.

REPORT OF COMMITTEE TO CONFER WITH AMERICAN PUBLIC HEALTH ASSOCIATION ON STANDARD METHODS FOR THE EXAMINATION OF DAIRY PRODUCTS

Your Chairman is pleased to report that the ninth edition of *Standard Methods for the Examination of Dairy Products*, to be published by the American Public Health Association, is in galley form and should be in print about January 1, 1948.

This edition contains 13 chapters, the first and last of which initiate a new policy. Chapter 1 is devoted essentially to explanatory matter for guidance of the less well informed administrator in the selection of tests to determine sanitary quality and in the interpretation of the results obtained by such tests.

Chapters 2 to 12 outline microbiological methods for milk, cream, butter, cheese, frozen dessert ingredients, and frozen desserts, and for the coliform group and certain pathogenic species of bacteria therein; also methods for sterility tests for dairy equipment, sediment and extraneous matter in dairy products, vitamin assays, phosphatase methods to determine pasteurization, and certain chemical procedures.

Chapter 13 bears the title "Screening Tests." It contains a selected group of procedures which permit determinations routinely on many more samples than could be made in case determinations were made by one or more of the refined technics. Where litigation may be elected, workers are warned not to depend upon the results obtained by screening tests. Where contests are expected, the methods should be such as will leave no doubt as either to the representative character of the sample or the dependability of the method used.

Representatives from Laboratory Committees in the American Public Health Association wish the members of this Association to know that they appreciate the priviledge granted to them to quote in the A.P.H.A. Methods certain sections from your Book of Methods.

Approved.

A. H. ROBERTSON, *Chairman*
GUY G. FRARY
J. O. CLARKE

REPORT OF COMMITTEE ON DEFINITIONS OF TERMS AND INTERPRETATIONS OF RESULTS ON FERTILIZERS

By L. S. WALKER, *Chairman*

*Official Final Action**

GUARANTEEING IN TERMS OF ELEMENTS

All fertilizer components with the exception of potash (K_2O) and phosphoric acid (P_2O_5), if guaranteed, shall be stated in terms of the elements.

Fused tricalcium phosphate is a product composed chiefly of the alpha form of the compound represented by the formula $Ca_3(PO_4)_2$. Its fineness and content of available phosphoric acid, (P_2O_5) shall be stipulated. Example: Fused tricalcium phosphate—twenty-five per cent (25%) available phosphoric acid (P_2O_5).

Calcium metaphosphate is a product composed chiefly of the vitreous compound indicated by the formula $Ca(PO_3)_2$. Its fineness and its content of available phosphoric acid (P_2O_5) shall be stipulated. Example: Calcium metaphosphate—sixty per cent (60%) available phosphoric acid (P_2O_5).

Potassium metaphosphate is a product composed chiefly of the crystalline compound represented by the formula KPO_3. Its fineness and content of phosphoric acid (P_2O_5) and of potash (K_2O) shall be stipulated. Example: Potassium metaphosphate—fifty-five per cent (55%) available phosphoric acid (P_2O_5): thirty-seven per cent (37%) potash (K_2O).

Double sulfate of potash and magnesia (Langbeinite). Double sulfate of potash and magnesia (langbeinite) is a commercial product containing not less than twenty-one per cent (21%) of potash (K_2O), nor less than fifty-three per cent (53%) of sulfate of magnesia and not more than two and one-half per cent (2.5%) of chlorine.

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* NOTE.—The above report was approved by vote of the Association with respect to the official, final action, definitions of terms and interpretation, and the proposed definitions. The recommendation that the Association of Official Agricultural Chemists discontinue the work on the Definition of Terms and the Interpretation of Results on Fertilizers and Liming Materials, after being duly moved and seconded for adoption, was laid on the table by a vote of 14 to 9.

PROPOSED DEFINITION

Fused Calcium-Magnesium Phosphate is a product derived from the fusion of rock phosphate with approximately thirty per cent (30%) of magnesium oxide (MgO), as such or as a mineral silicate. Its fineness and content of available phosphoric acid (P_2O_5) shall be stipulated. Example: Fused Calcium—magnesium phosphate, twenty per cent (20%) available phosphoric acid (P_2O_5).

Your committee has been asked by the Executive Committee to study the question of relinquishing the activities involved in the study of Definitions of Terms and Interpretations of Results on Fertilizers and Liming Materials. Anticipating this change, your chairman communicated with each member of the committee requesting an expression of views on this subject. The Committee has voted in favor of this change by a vote of four to two. It is therefore recommended that the Association of Official Agricultural Chemists discontinue the work on the Definitions of Terms and Interpretations of Results on Fertilizer and Liming Materials.

Approved.

REPORT OF THE COMMITTEE ON RECOMMENDATIONS OF REFEREES

WM. F. REINDOLLAR, *Chairman*

One of the chief duties of the Chairman of this Committee is to coordinate the reports of the referees and associate referees and to encourage and stimulate them to complete their assignment and to submit reports at a date sufficiently in advance of the meeting for them to receive due consideration by the proper subcommittee. During the past year this was accomplished by sending out three series of letters, one in the early part of the year, one in the spring, and the last in the late summer. While the response to these efforts has been rather gratifying, it was felt that an undue burden was placed on the referees by requiring them to send copies of their reports to several individuals. In order to standardize the procedure for handling reports and at the same time to minimize inconvenience to all concerned, the following plan will be followed in the future.

1. Associate referees are requested to prepare not less than six copies of their respective reports and mail them not later than a definite date which will be announced by direct correspondence.

2. All six copies are to be mailed to the office of the Secretary-Treasurer in Washington, D. C. The ribbon copy is to be included in this group unless the worker plans to be present and give his own paper, in which event it is to be turned in immediately after presentation. The associate referee does not have to mail copies to anyone else.

3. Upon receipt of the reports the Secretary-Treasurer will immediately mail one copy to each member of the appropriate subcommittee, one to the appropriate general referee, and one to the chairman of the Committee on Recommendations of Referees.

4. The Secretary-Treasurer will also keep these individuals posted as to which associate referees will have no reports or delayed reports.

5. General referees will be requested to review the work of their associates and to submit their own reports not later than a definite date which will be announced.

The chairman wishes to express his appreciation to the several subcommittees, referees, associate referees, and collaborators whose efforts have contributed so materially to the success of this Conference.

Approved.

REPORT OF SUBCOMMITTEE A ON RECOMMENDATIONS OF REFEREES*

By G. E. Grattan (Department of Agriculture, 79 Sussex St., Ottawa, Can.), *Chairman*; H. A. Halvorson, and E. L. Griffin

FEEDING STUFFS

It is recommended—

(1) That the following editorial corrections be made on page 419, method **27.59**, line 11. The following to be inserted before the words, "compare. . .$KMnO_4$" "Cool, make to measured volume of 50 or 100 ml, and mix."

(2) That further study be made on the following:

(a) Calcium and iodine in mineral mixed feeds.
(b) Lactose in mixed feeds.
(c) Adulteration of condensed milk products.
(d) Crude fat or ether extract.
(e) Activity of yeast.
(f) Fluorine.
(g) Protein evaluations in fish and animal products.
(h) Hydrocyanic acid glucosides.
(i) Sampling and analysis of condensed buttermilk.
(j) Microscopic examination of feeds.
(k) Tankage (hide, hoof, horn, and hair content).

(3) That the methods for calcium and phosphorus as outlined by the Associate Referee be made tentative and work continued.

(4) That the acetone method for fat in fish meal be adopted as tentative.

(5) That editorial changes in the method for crude fiber be made as recommended by the Associate Referee and the study continued.

(6) That the work on fat in cooked animal feeds be discontinued.

(7) That the method for nitrogen, **2.26**, be made preferential when applied to feeding stuff materials containing 30% or more of protein and that the work be discontinued.

FERTILIZERS

It is recommended—

(1) That line 6 of section **2.1**, *Methods of Analysis* (1945) be changed to read "If less than 100 bags, sample not less than 20 bags."

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* These recommendations submitted by Subcommittee A were approved by the Association. Unless otherwise given all references are to *Methods of Analysis, A.O.A.C.*, 1945.

That the following be added in section 2.1, line 7, before the final sentence: "In sampling a bulk lot of fertilizer draw not less than 20 cores from different regions of the lot. In sampling fertilizer in small containers (10 lbs or less) a single package may constitute a sample for the lot;" and the whole section be made official, first action, and the study of sampling equipment and methods be continued.

(2) That work on methods for phosphoric acid be continued, with emphasis on

(a) the ammonium citrate method and its applicability to basic slag;
(b) evaluation of sintered, fused, and calcined phosphates as fertilizers;
(c) the aging of molybdate solutions.

(3) that the recommendations of the Associate Referee on P_2O_5 be adopted and the methods be made official, first action.

(4) That attempts to work out improved methods for moisture be continued.

(5) That work on methods for nitrogen be continued with special emphasis on fertilizers which contain ammonium nitrate.

(6) That work on methods for manganese and magnesium be continued.

(7) That the study of problems relating to acid- and base-forming qualities of fertilizers be continued.

(8) That the work on potash be continued.

(9) That 95% ethyl alcohol be adopted instead of 80% alcohol in the determination of potash (official, first action).

(10) That work on analysis of sulfur be continued.

(11) That the study of methods for copper and zinc be continued.

(12) That the work on methods of analysis for boron be continued.

(13) That editorial correction in section 2.10(b) of "323.81" to "324.03" line 1 and of "32.38" to "32.40" line 2 be made.

ECONOMIC POISONS

It is recommended—

(1) That the method for total chlorine in emulsions containing DDT, solvent, emulsifying agent, and water, as recommended by the Associate Referee, be adopted as tentative.

(2) That the methods for DDT be amended as recommended by the Associate Referee and retained as tentative.

(3) That specific methods be sought for the determination of 1080 and Antu in rodenticides.

(4) That methods for fluorine and nicotine be discontinued.

(5) That studies on tetra ethyl pyrophosphate, benzene hexachloride, insecticides containing derris or cubé, oil emulsions, and herbicides be instituted.

(6) That the title of the section on Insecticides and Fungicides be changed to "Economic Poisons."

DISINFECTANTS

No report was received. It is recommended that the work be continued.

LEATHER AND TANNING MATERIALS

No report was received. It is recommended that the work be continued.

PLANTS

It is recommended—

(1) That the Associate Referee on copper and cobalt be relieved of the assignment as requested.

(2) That the Associate Referee on carbohydrates be relieved of his assignment as requested and the title changed to "sugar."

(3) That Associate Referees be appointed to study sodium, cellulose and lignin, pectin, and starch.

(4) That the other Associate Referees retain their assignments and continue their studies.

(5) That the studies on boron be continued.

(6) That the studies on zinc be continued.

SOILS AND LIMING MATERIALS

It is recommended—

(1) That studies on the "combination dithizone-spectrographic method" and on the polarographic procedure for the determination of zinc in soils be continued.

(2) That the study of the determination of copper in soils be continued.

(3) That the utilization of carmin as an indicator in the determination of boron content of soils be studied further and that p-nitrobenzeneazo-1, 8-dihydroxy naphthalene-3, 6-disulphonic acid, or "chromotrope-B" be studied as a suitable reagent for the determination of boron in soils.

(4) That further studies in *p*H in soils of arid and semi-arid regions be based upon soil systems of moisture content representative of an air-dry soil.

(5) That the analytical technic previously proposed for ignition of charge and distillation of fluorine therefrom be studied collaboratively.

(6) That "500" be substituted for "900" (p. 13, section **1.32,** line 3).

(7) That a study be made as to the adequacy of $Ca(OH)_2$ as a fixative for fluorine in soil charges of 1 to 1 proportion with calcination at 500 C in 5 to 60 minute periods.

(8) That the direct distillation of unignited soil with H_2SO_4 at 165°C with sequential distillation of an aliquot at 135°C be studied collaboratively.

(9) That the "2 point" titration procedure for the determination of exchangeable H in soils be studied further in relation to liming practice.

(10) That the survey and comparison of methods for the determination of phosphorus (a) that fraction in "available" state and (b) the propor-

tions of organic-inorganic forms therein be continued (*This Journal*, **30**, 43).

(11) That the survey and comparisons of methods for the determination of exchangeable K in soils be continued. (*This Journal*, **30**, 44).

(12) That the direct titration against bromcresol green (Method II, *This Journal* **30**, 297 (1947), be adopted as optional for the determination of the neutralizing value of blast furnace slags.

(13) That the tentative procedures in "Liming materials" be annotated by the statement "without correction for sulphide content."

(14) That the correction for sulphide content be studied further.

STANDARD SOLUTIONS

It is recommended—

(1) That the method for the preparation and standardization of sodium thiosulphate solution (**43.28**) be adopted as official, final action.

(2) That the method recommended by the National Bureau of Standards for the standardization of potassium permanganate solution be adopted as official, first action, and that it replace the official method appearing in the sixth edition, *Methods of Analysis*.

(3) That the method for the preparation and standardization of bromide-bromate solutions be adopted as official, first action.

(4) That the methods for the standardizing of titanium trichloride solutions be studied further.

(5) That the study on buffer solutions be continued.

(6) That the study of the preparation and standardization of potassium dichromate solutions be continued.

(7) That the method for the preparation and standardization of thiocyanate solutions be adopted as official, final action.

VITAMINS

It is recommended—

(1) That the collaborative procedure for the determination of carotene be studied further, with different samples of alfalfa meal and other types of food and feed materials.

(2) That the collaborative procedure as outlined for the determination of carotene in hays and dried plants be adopted as an alternate tentative method.

(3) That the General Referee consider the question of recommending the inclusion of both saturated steam pressure and temperature for sterilizing, in view of the disagreement in the *U.S. Pharmacopoeia XII* and the *Handbook of Chemistry and Physics* (19th Ed.).

(4) That the thiochrome method for thiamine (**36.24–36.26** incl.) be made official (first action).

(5) That further work be conducted on the fluorometric method for

vitamin B_2 and that further consideration be given to the present microbiological method.

(6) That the method for folic acid be given further study.

(7) That the other vitamin methods for which there were no reports be given further study.

REPORT OF SUBCOMMITTEE B ON RECOMMENDATIONS OF REFEREES*

By H. J. Fisher (Agricultural Experiment Station, New Haven, Connecticut), *Chairman*; G. R. Clark, and F. H. Wiley

NAVAL STORES

No report was received; it is recommended that the subject be continued.

RADIOACTIVITY

No reports were received. It is recommended that studies on various methods of measurement of radioactivity be undertaken as Associate Referees are available.

SPECTROGRAPHIC METHODS

The Referee submitted a report outlining the quantitative technique used in his laboratory for the determination of metals and phosphorus in plant materials and illustrating the precision that had been obtained. He recommends that samples of various types of plant materials be submitted to collaborators for analysis by their own techniques and that these techniques then be compared as to their relative accuracy. At a later date the one technique found to be most promising would be submitted to all collaborators for further study. The committee concurs in these recommendations.

VEGETABLE DRUGS AND THEIR DERIVATIVES

It is recommended—

(1) That the study of chemical methods for ergot alkaloids be continued.

(2) That the study of methods for determining physostigmine be continued, with particular attention being paid to the determination of this alkaloid in ointments.

(3) That the study of the determination of theobromine and phenobarbital in mixtures be continued.

(4) That the study of the separation of aminopyrine, ephedrine, and phenobarbital be continued.

(5) That the study of the determination of quinine by the Herd and other methods be continued.

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* These recommendations submitted by Subcommittee B were approved by the Association. Unless otherwise given all references are to *Methods of Analysis, A.O.A.C.*, 1945.

(6) That the method proposed by the Associate Referee for the determination of ephedrine be adopted as official (first action) and the subject closed.

(7) That the modification of the N. F. VIII method for the determination of camphor in spirit of camphor proposed by the Associate Referee be adopted as official (first action) and also referred to the Committee on the National Formulary, and that the subject be closed.

(8) That the study of chemical methods for penicillin be continued.

(9) That a study be made of the determination of rutin in tablets.

(10) That a study be made of the determination of ethylmorphine in syrups.

SYNTHETIC DRUGS

It is recommended—

(1) That study of the determination of methylene blue be continued, with particular attention being paid to spectrophotometric methods.

(2) That the study of methods for the determination of sulfanilamide derivatives be reassigned.

(3) That the method proposed by the Associate Referee for the determination of atabrine (quinacrine hydrochloride) be adopted as official (first action) and the topic closed.

(4) That the distillation and extraction methods for demerol previously outlined by the Associate Referee [*This Journal*, **28**, 712 (1945)] be adopted as official (first action) and the subject closed.

(5) That the study of the determination of propadrine hydrochloride be continued.

(6) That the study of the estimation of carbromal by the determination of bromine be continued.

(7) That the method proposed by the Associate Referee for the determination of dihydrocodeinone be adopted as official (first action) and the subject closed.

(8) That the study of butacaine sulfate be continued.

(9) That the study of spectrophotometric methods for drugs be continued.

(10) That the study of the determination of trichloroethylene be continued, with particular attention being paid to the Rauscher method for chlorine in organic compounds.

(11) That the method proposed by the Associate Referee for the determination of thiouracil in tablets be adopted as official (first action) and the propyl derivative of this compound studied next year.

(12) That the study of methods for the determination of phenolphthalein in chocolate preparations be continued.

(13) That a study be made of the detection and determination of pyribenzamine and benadryl.

(14) That a study be made of the determination of synthetic estrogens.

MISCELLANEOUS DRUGS

It is recommended—

(1) That the title of the Associate Refereeship for microchemical tests for alkaloids and synthetics be changed to "Microscopic tests for alkaloids and synthetics," and the subject continued.

(2) That study of the ethanolamine method for mercury be continued.

(3) That study of the separation of chlorides, bromides, and iodides be reassigned.

(4) That the Associate Refereeship on organic iodides be discontinued and work on this subject be combined with that on the separation of the halogens.

(5) That the method proposed by the Associate Referee for the assay of compound ointment of benzoic acid be adopted as official (first action) and the topic closed.

(6) That study of the separation of the alkali metals be continued.

(7) That study of the determination of glycols and related compounds be continued.

(8) That study of methods for preservative and bacteriostatic agents in ampul solutions be continued.

(9) That study of the determination of phosphorus, calcium, and iron in vitamin preparations be continued.

(10) That the recommendation of the Associate Referee that his proposed modification of the method for total iodine be submitted to collaborative study be approved.

(11) That the subject of superheated steam in separation of drugs be dropped.

(12) That the study of estrone and estradiol be continued.

(13) That the study of the chromatographic separation of drugs be continued.

(14) That the tentative method for methyl alcohol, sections **39.161–39.162,** be studied, together with the directions in **16.25,** to bring about uniformity in these procedures.

DRUG BIOASSAYS

No reports were received. Since it appears to be impractical, if not impossible, for the Association to obtain qualified collaborators for work of this sort, it is recommended that the Refereeship on drug bioassays be abolished.

COSMETICS

It is recommended—

(1) That the periodate consumption and formic acid titration methods proposed by the Associate Referee be made official (first action); and study of other cosmetic cream problems be undertaken.

(2) That the following topics on which no reports were received be continued:

Alkalies in cuticle removers
Hair straighteners
Cosmetic powders
Cosmetic skin lotions
Deodorants and antiperspirants
Depilatories
Hair dyes and rinses
Mercury salts in cosmetics

(3) That the Associate Referee's recommendation of further study of methods for mascara, eyebrow pencils, and eye shadow be approved.

(4) That the study of the determination of moisture in cosmetics be continued.

(5) That the study of the determination of pyrogallol in the presence of henna be continued.

COAL-TAR COLORS

(6) That the following topics on which no reports were received be continued:

Acetates, carbonates, halides, and sulfates in certified coal-tar colors
Buffers and solvents in titanium trichloride titrations
Ether extract in coal-tar colors
Identification of certified coal-tar colors
Unsulfonated phenolic intermediates in coal-tar colors
Mixtures of coal-tar colors for drug and cosmetic use

(7) That the following topics on which no reports were received be continued and reassigned:

Halogens in halogenated fluoresceins
Nonvolatile unsulfonated amine intermediates in coal-tar colors
Sulfonated amine intermediates in coal-tar colors
Intermediates derived from phthalic acid
Sulfonated phenolic intermediates in coal-tar colors

(8) That the method proposed by the Associate Referee for the determination of aniline in coal-tar colors be adopted as official (first action), and that study of the applicability of this method to other volatile amines be continued.

(9) That adoption of the method proposed by the Associate Referee for the determination of pure dye in lakes and pigments be deferred until the method has been tested on other dyes.

(10) That study of the following topics be continued as recommended by the Associate Referees:

Spectrophotometric testing of coal-tar colors
Subsidiary dyes in D&C colors

(11) That the following new topics be studied:

Lead in coal-tar colors
Lead in lakes of coal-tar colors

Subsidiary dyes in FD&C colors
Hygroscopic properties of coal-tar colors

REPORT OF SUBCOMMITTEE C ON RECOMMENDATIONS OF REFEREES*

By Joseph Callaway, U. S. Food and Drug Administration, Washington, D. C., *Chairman;* Paul A. Clifford, and A. H. Robertson

PROCESSED VEGETABLE PRODUCTS

It is recommended—

(1) That studies of methods for determining quality factors in canned and frozen fruits and vegetables be continued.

(2) That studies of the determination of a moisture in dried vegetables be continued.

(3) That studies of methods for the estimation of the enzymatic activity in frozen fruits and vegetables be continued.

FILL OF CONTAINER METHODS

It is recommended—

(1) That studies of methods for determining fill of container for foods, drugs, and cosmetics be continued.

COFFEE AND TEA

It is recommended—

(1) That the Referee review the chapter on coffee and tea and make recommendations for additional work where this appears desirable.

COLORING MATTERS IN FOOD

It is recommended—

(1) That the method for the detection of small amounts of tartrazine in alimentary paste as modified by the Associate Referee be made official, first action.

(2) That work be continued on the separation and quantitative determination of tartrazine (FD&C No. 5) and sunset yellow FCF (FDC No. 6).

(3) That investigational work be undertaken to separate and determine quantitatively FD&C Green No. 2 (Light Green S.F. yellowish), FD&C Green No. 3 (Fast Green F.C.F.), and FD&C Blue No. 1 (Brilliant Blue F.C.F.).

(4) That investigational work be undertaken to separate and estimate quantitatively FD&C Yellow No. 3 (Yellow A.B.), FD&C Yellow No. 4 (Yellow O.B.), FD&C Orange No. 2 (Orange S.S.), and FD&C Red No. 32 (Oil Red X.O.).

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* These recommendations submitted by Subcommittee C were approved by the Association. Unless otherwise given all references are to *Methods of Analysis, A.O.A.C.*, 1945.

DAIRY PRODUCTS

It is recommended—

(1) That the Sanders-Sager method for phosphatase in fluid milk and cream, in cheddar type cheese, and in soft uncured cheeses, be made official, first action. That this test with modification making it applicable to other designated types of cheese, ice cream mix, sherbet mix, chocolate-flavored milks and skim milks, butter, sweet cream and cultured buttermilks, fermented milks, goats' milk, cheese whey, and concentrated milks, be adopted as tentative and subjected to collaborative tests; and that the present tentative methods for phosphatase (**22.43** to **22.57**) be dropped.

(2) That studies of methods for the detection of reconstituted milks be continued.

(3) That studies of methods for the detection of chlorine added to milk be continued.

(4) That the rapid screening method for moisture described by the Associate Referee be adopted as tentative, and that methods for the preparation of samples of various types of cheese, processed cheeses, and related products, and methods for the determination of fat in these foods, be further studied.

(5) That studies of methods for determining the acidity of milk be continued.

(6) That the use of mechanical shaking devices and of artificial methods of cooling suitable for use in the present official method for the preparation of butter samples be studied and that the mechanical stirrer method described by the Associate Referee be adopted as tentative.

(7) That methods for the determination of ash in milk and evaporated milk be further studied.

(8) That the official method (**22.146b**) for the preparation of samples for frozen dessert and other methods for the preparation of samples be further studied.

(9) That an associate referee be appointed to study and make recommendations for methods for the determination of various fruits and other characterizing ingredients of ice cream and other frozen desserts.

(10) That studies be initiated to ascertain whether the present Babcock method for fat in milk should be modified when used for determining fat in homogenized milk.

(11) That the Roese-Gottleib method in dairy products be studied.

(12) That the editorial changes in wording recommended by the Referee in section **22.149** (fat in ice cream) be made.

(13) That there be inserted in sections **22.28 (a)**, **22.29**, and **22.30** directions for converting readings made on Bausch and Lomb instruments to corresponding readings on Zeiss instruments, and that a study of these sections be continued.

(14) That the test for sediment in fluid milk (**22.40, 22.41,** and **22.42**) be dropped and that the American Public Health Association method for sediment in fluid milk be adopted as tentative and further studied.

(15) That the editorial changes in the wording of section **22.33** recommended by the Referee be made.

EGGS AND EGG PRODUCTS

It is recommended—

(1) That the method for the determination for added glycerol in liquid and frozen eggs, and egg yolks, be further studied.

(2) That the method for the acidity of the ether extract of dried egg and dried egg yolk proposed by Kline and Johnson be further studied, utilizing if possible samples of dried egg products of known history.

MICROANALYTICAL METHODS FOR EXTRANEOUS MATERIALS IN FOODS AND DRUGS

It is recommended—

(1) That the method for rodent excreta in corn meal (**42.32**) be made official, first action.

(2) That methods for extraneous matters in fruits be subjected to collaborative studies.

(3) That methods for extraneous matters in drugs be further studied.

(4) That the changes in wording of section **42.57** (mold in tomato products) providing for adjusting the total solids in the sample by the use of the Abbe refractometer, as recommended by the Associate Referee, be made.

(5) That the changes in wording of section **42.11** (manure fragments in dairy products), recommended by the Referee, be made.

DECOMPOSITION IN FOODS

It is recommended—

(1) That the method described by the Associate Referee for the determination of indole in shrimp, oysters, and crabmeat be adopted as tentative and work on other indices of decomposition in these foods be continued.

(2) That the method for the determination of galacturonic acid in apple products described by the Associate Referee be further studied and that substances other than galacturonic acid be investigated as indices of the use of rotten apples in the manufacture of apple products.

(3) That studies be continued on fish and dairy products.

GELATIN, DESSERT PREPARATIONS, AND MIXES

It is recommended—

(1) That changes in the method for the determination of jelly strength in gelatin (**9.6**) recommended by the Associate Referee, be made; and that this method as revised and also the method for determination of jelly strength in dessert powders (**9.12**) be subjected to collaborative study.

FISH AND OTHER MARINE PRODUCTS

It is recommended—

(1) That the composition of the substances extracted by ether and by mixtures of ether and petroleum ether after the acid hydrolysis of the fish be further studied, with the idea of determining whether the material extracted is largely fat, before final adoption of this method for the determination of fat in fish.

(2) That work be continued on selecting a suitable method for determining total solids in fish, and, if practicable, that such method be subjected to collaborative study.

GUMS IN FOOD

It is recommended—

(1) That the method for detection of gums in soft uncured cheeses be further studied as to its quantitative application and as to whether it detects the presence of soluble alginates.

(2) That studies be continued on the detection of gums in cacao products.

(3) That studies be continued on the detection and determination of gelatin gums and other stabilizers in frozen desserts.

(4) That studies be continued on the detection of gum in mayonnaise, French dressing and related salad dressings.

MEAT AND MEAT PRODUCTS

It is recommended—

(1) That work be continued on the development of methods for the determination of soya flour in meat products.

(2) That work be initiated on the development of a method for the determination of nonfat dry milk solids in meat products.

(3) That studies be made of additional methods for the determination of creatin in meat products.

METALS, OTHER ELEMENTS, AND RESIDUES IN FOODS

It is recommended—

(1) That methods for the determination of cadmium be further studied.

(2) That the methods for the determination of copper of Greenleaf, and of Bendix and Grabenstetter, be subjected to collaborative study.

(3) That methods for the determinations for DDT in spray residue and canned foods be further studied.

(4) That methods for the determination for mercury in spray residue be further studied.

(5) That methods for the determination of zinc be further studied.

MICROBIOLOGICAL METHODS

It is recommended—

(1) That work be continued on eggs and egg products, sugar, canned

vegetables, canned meat products, canned fish, acid canned foods, nuts and nut products.

(2) That methods for the examination of frozen fruits and vegetables outlined by the Associate Referee be studied and if practicable be subjected to collaborative study.

MICROCHEMICAL METHODS

It is recommended—

(1) That the collaborative study of microchemical methods be undertaken for carbon and hydrogen, and the Dumas and Kjeldahl methods for nitrogen.

NUTS AND NUT PRODUCTS

It is recommended—

(1) That methods for the preparation of samples and the determination of moisture and fat in nuts and nut products be further studied.

OILS, FATS, AND WAXES

It is recommended—

(1) That the method for unsaponifiable matter (**31.40**) be made official, final action.

(2) That a chromatographic procedure for the purification of the unsaponifiable matter be studied.

(3) That the F.A.C. Method for unsaponifiable matter (**31.37** to **31.39** inclusive) be deleted, final action.

(4) That the method for squalene (**31.41–31.43**) be made official, final action.

(5) That studies of methods for determining the stability of fats be discontinued for the present.

(6) That studies of methods for the estimation of peanut oil be continued.

(7) That studies on methods for the detection and determination of antioxidants in fats be continued.

PRESERVATIVES AND ARTIFICIAL SWEETENERS

It is recommended—

(1) That work on the esters of benzoic acid be continued and if practicable that methods also be found for the detection and determination of esters of vanillic acid.

(2) That methods for the quantitative determination of saccharin in baked goods be further studied.

(3) That the method for saccharin described by the Associate Referee *This Journal*, **30**, 492 (1947), be further studied.

(4) That the method for estimation for volatile fatty acids in bakery products described by the Associate Referee be adopted as tentative and

work on this subject continued with the idea of utilizing if possible the chromatographic procedure for the separation of propionic acid.

(5) That the tests for the detection of thiourea in orange juice, described by the Associate Referee, be adopted as tentative and work continued. That the quantitative methods for thiourea in orange juice and frozen peaches, described by the Associate Referee, be adopted as tentative and the work continued.

(6) That work be continued on methods for the detection and estimation of dulcin in foods.

(7) That work be initiated on the detection and estimation of the artificial sweetener having the composition 1-propoxy 2-amino 4-nitrobenzene.

(8) That work be initiated on the detection and determination of dichloracetic acid in foods.

(9) That work be continued on the development of improved methods for the detection of formaldehyde in foods and that unnecessary and unused methods now carried as official method be recommended for deletion.

(10) The the method for the determination for monochloracetic acid (applicable to carbonated beverages and fruit juice, *This Journal,* **27,** 199, 1944), with the provision as to the use of alternate method of extraction recommended by the Associate Referee, be made official, first action; that further collaborative work be done on other fruit juices and beverages and that the application of this method to other foods be studied; and that collaborative work on the indigo and pyridine qualitative tests for monochloracetic acid be continued.

(11) That the ferricyanide method for quaternary ammonium compounds in commercial preservatives, *This Journal,* **29,** 312 (1946), be made official, first action; that the method for the determination of quaternary ammonium compound in table syrup, *This Journal,* **29,** 325 (1946), be made official, first action; that the method for the determination of quaternary ammonium compound in beverages containing fruit juices, *This Journal,* **29,** 322 (1946), be made official, first action; that the method for the determination of quaternary ammonium compounds in beer, *This Journal,* **29,** 325 (1946), be made official, first action; and that studies be continued of methods for the determination of quaternary ammonium compounds in fruit juices, soda waters, milk, salad dressings, sandwich spreads, pickles, relishes, and related products.

SPICES AND CONDIMENTS

It is recommended—

(1) That the method for the determination of caramel in vinegar **(33.78)** be dropped and that studies be made of other tests for caramel, such as Mathers test, and the test for caramel in wine **(15.39),** to determine their applicability to the detection of caramel in vinegar.

(2) That the permanganate oxidation method be applied to a number of samples of distilled vinegars of known history in order to ascertain if it has value in differentiating this type of vinegar from a dilute acetic acid.

(3) That the methods for the quantitative determination of free mineral acids in vinegar be further studied.

(4) That studies of the determination of tartaric acid and tartrates in vinegar be continued.

(5) That the method, as recommended by the Associate Referee for starch in mayonnaise and salad dressing, be made official, first action, and that work be initiated looking toward the adoption, as official, of methods for the preparation of samples and determination of total fat in mayonnaise and salad dressing.

(6) That the present tentative method for starch in prepared mustard and mustard flour, modified as suggested by the Associate Referee, be adopted as official, first action.

(7) That studies be initiated looking toward adoption of a method for the determination of ash in prepared mustard.

(8) That the official method for copper-reducing substances **(33.40)** be dropped, final action.

(9) That studies be initiated looking toward adoption of a method for the determination of sugars in prepared mustard.

(10) That studies be continued of methods for the determination of volatile oils and other pungent principles in prepared mustard and mustard flour.

(11) That studies be continued for methods for the determination of volatile oils in spices.

REPORT OF SUBCOMMITTEE D ON RECOMMENDATIONS OF REFEREES*

By C. S. Ferguson, State Department of Public Health, Boston, Mass., *Chairman;* Kenneth L. Milstead, and J. Walter Sale

ALCOHOLIC BEVERAGES

Malt Beverages, Brewing Materials, and Allied Products:

It is recommended—

(1) That the following official (first action) methods for malt be adopted as official, final action: Sampling **(14.39)**; Preparation of sample **(14.40)**; Bushel weight **(14.41)**; 1,000 Kernel weight **(14.45)**; Moisture in caramel malt and black malt **(14.52)**; Diastatic power **(14.61)**.

(2) That the following official (first action) methods for beer be adopted as official, final action: Extract of original wort **(14.7)**; Real degree of

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* These recommendations submitted by Subcommittee D were approved by the Association. Unless otherwise given all references are to *Methods of Analysis, A.O.A.C.*, 1945.

fermentation or real attentuation (**14.8**); Apparent degree of fermentation or apparent attenuation (**14.9**); Total acidity—Indicator Titration method (**14.10**); Total Acidity—potentiometric titration method (**14.11**); H-ion concentration (*p*H)—Electrometric method (**14.12**).

(3) That the following official (first action) methods for hops be adopted as official, final action: Sampling (**14.80**); Physical examination (**14.81**); Preparation of sample for chemical analysis (**14.82**); Moisture (**14.83**); Resins (**14.84–14.89**, inclusive).

(4) That the following official (first action) methods for brewing sugars and syrups be adopted as official, final action: Extract (**14.90**); Non-extract (apparent water) (**14.92**); Fermentable extract—(b) Rapid Fermentation method (**14.92**); Protein (**14.93**); Iodine reaction for unconverted starch (**14.95**); Acidity (**14.96**); H-ion concentration (*p*H) (**14.97**); Ash (**14.98**); Total reducing sugars—Munson-Walker general method (**14.99**).

(5) That the following official, first action methods for wort be adopted as official, final action: Specific gravity (**14.103**); Original extract or original gravity (**14.104**); Fermentable extract (**14.105**); Iodine reaction (**14.106**); Total acidity (**14.107**); H-ion concentration (*p*H) (**14.108**); Color (**14.109**); Protein (**14.110**); Total reducing sugars (**14.111**).

(6) That study of methods for determination of essential oil in hops be continued.

(7) That the study of the tentative method for color (*This Journal*, **30**, 68, 1947), and the photoelectric beer color evaluation, as well as work on beer turbidity methods, be continued.

(8) That the direct (non-ashing) orthophenanthroline method described in Proceedings of the Eleventh Annual Meeting of the American Society of Brewing Chemists, pages 32 and 37, for the determination of iron in beer, be studied collaboratively.

(9) That study of methods for testing soluble starches used in diastatic power determination of malt be discontinued.

(10) That study of methods for determination of total solids and yeast solids be continued in accordance with this year's report of the Associate Referee.

(11) That the Milos test for caramel, **14.35** be deleted, first action.

(12) That the Mathers test for caramel, described in this year's report of the Associate Referee on Wine, be studied collaboratively with respect to its application to beer.

(13) That the study of carbon dioxide in beer be continued.

(14) That the official modified Denigés' method for methanol, section **16.25**, be studied together with the directions in **39.161–39.162**, to bring about uniformity in these procedures.

Wines:

It is recommended—

(1) That study of the spectrophotometric examination of wines be discontinued.

(2) That chromatographic studies in wines be continued.

(3) That study of methods for methanol in wines and distilled liquors be continued.

(4) That the official Milos test for caramel (**15.38**) be deleted (first action).

(5) That the Mathers test, as described in the report of the Associate Referee this year, be adopted as official, first action.

(6) That the tentative confirmatory test for caramel (**15.39**) be modified as described in the report of the Associate Referee for this year.

Distilled Liquors:

It is recommended—

(1) That the study of the obscuration method for determining the true proof of blended spirits be continued.

(2) That the study of methods of analysis with reference to the aging or maturing of whiskey in laminated (plywood) barrels be continued.

(3) That method **16.46 (b)** be changed as recommended by the Associate Referee in this year's report and that study of cordials and liqueurs be continued.

(4) That the official modified Marsh test and the official Milos test, **16.39** and **16.41**, for caramel, be deleted (first action).

(5) That the Fulton test for caramel, described in this year's report of the Associate Referee, be adopted as tentative, for distilled liquors and for cordials and liqueurs.

(6) That the Mathers test for caramel, described in this year's report of the Associate Referee, be adopted as official (first action) for Distilled Liquors.

(7) That the Mathers test for caramel, described in this year's report of the Associate Referee, be adopted as official (first action) for cordials and liqueurs, and that it be included by reference in the chapter on cordials and liqueurs in **16.60**, in place of the modified Marsh test, **16.39**, and the Milos test, **15.38**.

(8) That an Associate Referee be appointed to study a rapid colorimetric method for fusel oil.

(9) That the method for fusel oil, **16.22** (p. 196) be further studied.

CACAO BEAN AND ITS PRODUCTS

It is recommended—

(1) That the work on lecithin (*This Journal*, **25**, 717) be continued.

(2) That the work on the determination of maltose and of lactose in the presence of other reducing sugars be continued.

(3) That the method for tannins and pigments in this year's Referee Report, be adopted as tentative.

(4) That the three procedures for fat in refractory beverage bases, described in this year's report of the Associate Referee, be further studied.

(5) That work be continued on the tentative method for pectic acid, **19.16**, with particular reference to milk chocolate.

CEREAL FOODS

It is recommended—

(1) That both procedures proposed by the Associate Referee for the determination of phosphorus in cereals and cereal products be adopted as official (first action) and that the study be continued.

(2) That the dry ashing method for iron **20.9–20.12**, inclusive, be made official, final action, for enriched macaroni products, degerminated, bolted, and whole corn meals, and that the study be discontinued.

(3) That the wet ashing method for iron (*This Journal*, **30**, 71, 1947), be made official, final action, and that study be discontinued.

(4) That the studies on determination of starch in raw and cooked cereals be continued.

(5) That the tentative method for the determination of fat acidity in grain, flour, corn meal, and whole wheat flour (**20.18–20.21**, inclusive) be further studied and also that the relationship of acidity to unsoundness be studied.

(6) That the Associate Referee continue his work reported this year on the application of the method for reducing and non-reducing sugars in flour **20.28–20.30**, inclusive, to the determination of sugars in bread and other bakery products, with special consideration to the article on this subject published by R. M. Sandstedt and G. C. Fleming (*This Journal*, **30**, 550–2).

(7) That the tentative method for benzoyl peroxide in flour, **20.53**, be continued for rye flour and the title changed to Benzoic Acid in rye flour; and that the method proposed by the Associate Referee replace it for wheat flour.

(8) That work be continued on methods for determination of available CO_2 in self-rising flour containing added $CaCO_3$.

(9) That the method for the determination of lactose in bread (*This Journal*, **24**, 630) be further studied.

(10) That the determination of milk fat in bread, **20.86**, be further studied.

(11) That the methods for the determination of proteolytic activity of flour be continued.

(12) **(a)** That the method for moisture in soy flour, **20.77**, may be changed to read—"Moisture—*see* **20.2** or **20.4**, with the exception that a 5 g sample be dried 130° for 2 hours." **(b)** That the method for ash, **20.78**, be changed to read—"Ash—*see* **27.9**." **(c)** That the method for nitrogen, **20.79**, be changed to read—"Proceed as directed under **2.26**,

using 10 g K_2SO_4, or Na_2SO_4 and 0.7 g of HgO or its equivalent in Hg, with the additional option of using sodium alizarin sulfonate." **(d)** The the method for oil, **20.82,** be changed to read "*See* **31.07** except that ca 2 g full-fat flour or 5 g low or defatted soy flour be extracted for 5 hours" and that the study be continued on these methods.

(13) That studies be made on the detection and determination of soy bean flour in cereal products.

(14) That the method proposed by the Associate Referee for determination of the amount of added inorganic material in phosphated and self-rising flour be further studied.

(15) That the method referred to in *This Journal* **25,** 83–4, for the determination of unsaponifiable matter and sterols in noodles be studied to determine their applicability to other foods containing eggs.

(16) That studies of methods for the determination of albumin in noodles and other farinaceous egg-containing products be continued.

(17) That the method for the determination of total solids, **20.84(b),** for raisin bread and bread containing raisins and fruits (official, first action, *This Journal,* **30,** 72, 1947) be adopted as official, final action, and that study on the determination of moisture, fat, crude fiber, ash, and protein in bakery products be continued.

(18) That the study on the determination of moisture in all flour-like products containing sodium bicarbonate as one of its constituents be continued.

(19) That the study on the determination of bromates in flour be continued along the lines suggested in this year's report of the Associate Referee.

(20) That the study on the determination of apparent viscosity measurements of flour be discontinued.

BAKING POWDER

It is recommended—

(1) That the present official method for determination of residual carbon dioxide in baking powders be deleted (final action under suspension of the rules), for the following reasons:

(a) The wording and description of the method is susceptible to too many interpretations.

(b) The manner of heating and treating the sample does not yield a true residual because all of the available CO_2 is not driven off.

(c) The method is not applicable to all types of baking powder, particularly the pyrophosphate types.

(2) That the modified Quartermaster Corps method as described in the report of the Associate Referee this year, be adopted as a tentative method, adding the words "or electricity" after the phrase "The bath should be heated with gas."

(3) That the modified Q.M.C. method referred to in recommendation (2) be subjected to further study wherein the single evaporation to dryness is carried out in a moisture oven instead of on a water bath and at temperatures ranging from 70 to 100°C.

(4) That the modified Q.M.C. method referred to in recommendation (2) also be studied using a neutral saturated sodium chloride solution as a medium instead of distilled water.

(5) That the expression "or subtract 17.8 from 17.6" in method **17.9**, available carbon dioxide—official, be deleted, first action, and that the tentative available CO_2 be determined by subtraction of the tentative residual CO_2 from the total CO_2, official 17.6.

(6) That an investigation be made on modifying the present A.O.A.C. gravimetric method, **17.2** and **17.3**, by changing from H_2SO_4 and KOH absorption bulbs to the use of "Caroxite" or "Ascarite and Anhydrone."

FLAVORS AND NON-ALCOHOLIC BEVERAGES

It is recommended—

(1) That the collaborative study of the reflux method for determination of peel oil in citrus fruit juices and the use of the modified oil separation trap be continued.

(2) That collaborative work be continued on the method for determination of beta-ionone where small amounts are present.

(3) That collaborative studies on the Ripper method for determination of aldehydes in spirts as applied to lemon oils and extracts be continued.

(4) That collaborative studies of the methods proposed by the Referee for determination of esters in lemon extract be continued.

(5) That collaborative studies on the Seeker-Kirby Method for determination of esters in lemon and orange oils (Dept. of Agri. Bull. 241) be continued.

(6) That collaborative studies of extract methods containing both isopropyl alcohol and acetone be continued.

(7) That collaborative study of the photometric method for determination of vanillin and coumarin be continued.

(8) That work be continued on the determination of glycerol, vanillin, and coumarin in vanilla and imitation vanilla extracts with special reference to the automatic extraction of vanillin and coumarin.

(9) That the study of emulsion flavors be continued.

(10) That studies on maple concentrates and imitations be continued.

(11) That study of the method for determination of diacetyl, published in *This Journal*, **25**, 255, be continued.

(12) That section **25.2** be changed to read as follows:

"**25.2** Alcohol-Official—Proceed as directed in **16.6** or **16.7** but measure the sample used at 15.56°C. in a pycnometer (see pages 192–3) calibrated at that temperature."

(13) That the Referee study collaboratively the modification of **25.23** as given in his report.

(14) That the Referee study collaboratively the modification of **25.54** as given in his report.

(15) That **25.64** be changed to read as follows:

"**25.64** Alcohol-Official—Proceed as directed in **16.6** (b).

FRUITS AND FRUIT PRODUCTS

It is recommended—

(1) That study of methods for determining fruit and sugar content of frozen dessert fruits be continued.

(2) That further collaborative study be made of the method for electrometric titration of acidity.

(3) That further study be made of methods of separating and determining fruit acids.

(4) That the study of methods for the determination of water insoluble solids be continued.

SUGARS AND SUGAR PRODUCTS

It is recommended—

(1) That the Java method (*This Journal*, **29**, 242) for unfermented reducing substances in molasses be adopted as tentative, and that work be continued.

(2) That the study for the determination of moisture be continued.

(3) That study be continued on tables of density of sucrose solution at various temperatures.

(4) That the Zerban and Martin values for refractive indices of dextrose and invert sugar solutions (*This Journal*, **27**, 295) be subjected to collaborative study.

(5) That the study of the applicability of electro-deposition to the direct quantitative determination of dextrin in honey and honeydew honey be discontinued.

(6) That the study of the characteristic properties of dextrins of honey and honeydew honey be discontinued.

(7) That the official method for the determination of free acid in honey, **34.99**, be studied collaboratively with a view to establishing the end point more accurately.

(8) That study be made of methods for the detection of adulterants in honey, particularly commercial syrups.

(9) That collaborative study of the method described in last year's report of the Associate Referee, for the determination of resinous glaze in confectionery be continued, with special reference to large amounts of lac.

(10) That study be continued on the determination of dextrose, maltose, and dextrins, by copper reduction methods in pure sugar mixtures.

(11) That the tentative methods, **34.133–34.155**, inclusive, be subjected to collaborative study.

(12) That the procedures in N.B.S. Circular C440, pp. 324–334, for measurement of transmission of solutions of commercial sugar products, be considered with a view to their future adoption as tentative methods.

(13) That the Somogyi Modification (*J. Biol. Chem.*, V. 160, p. 61, 1945) of the tentative micro method for reducing sugars (**34.63**) be studied.

(14) That changes in accordance with the International Sugar Scale be adopted as official, final (special action) in sections **34.18** and **34.19.**

WATERS, BRINE, AND SALT

It is recommended—

(1) That studies of methods for the determination of borate be continued.

(2) That methods for determination of fluoride in salt be studied collaboratively.

REPORT OF A.O.A.C. REPRESENTATIVES ON THE BOARD OF GOVERNORS OF THE CROP PROTECTION INSTITUTE OF THE NATIONAL RESEARCH COUNCIL

The Crop Protection Institute congratulates the A.O.A.C. on its record of 61 years of very worth-while accomplishments, and sends greetings and best wishes for a continuation of the successful and fine record of the past and may the future have still better things in store for your useful organization.

The Board of Governors of the Crop Protection Institute continues to hold at least two meetings a year to discuss the progress of projects and consider new projects. The various project committees are always working and the Chairman of Board is perpetually active.

In the early years of the Institute, the work was largely with inorganic compounds. In recent years the material to be tested for crop protection are largely synthetic organic compounds. The progress which has been made in many fields are marvels of efficiency and in directions which were undreamed of a few years ago. Great progress has been made in developing organic insecticides and fungicides which are harmless to man and animals. Sprays have been developed which will prevent fruit from dropping from the trees. Much has been accomplished in developing weed killers with selective characters. So now lawns can be freed of objectionable weeds without injury to the grass. Much work has been done and much accomplished in protecting man and animals from objectionable and disease carrying insects and plants.

While much progress has been made in developing synthetic organic products for the control of objectionable plants, insects, and animals, though this field is in its infancy it offers a great opportunity for the research worker. This Association should encourage its members to promote this field of research and cooperate whenever possible with the entomologist, botanist, and pathologist in solving their problems.

The Crop Protection Institute has about the same number of projects under way as in former years. The numbers of people or firms who come to the Institute for help and research are increasing. The Institute continues the policy of cooperating with State experiment stations and university laboratories whenever possible. There is no doubt but that commercial industries need and appreciate having a neutral agency like the Crop Protection Institute to assist them in developing and testing their products.

We recommend that A.O.A.C. continue its active cooperation with the crop Protection Institute and direct its way such commercial work, of a more or less private character, as it is prepared to handle.

H. J. Patterson
W. H. MacIntire

REPORT OF COMMITTEE ON CLASSIFICATION OF METHODS

Your Committee has made a careful survey of all methods in the 1945 Edition of the Association's "Methods of Analysis."

Those methods clearly designated "Official" need no comment, other than to urge that any methods which have been for some time in the "First Action" status should be promptly adopted as Official, Final Action. If doubt has arisen as to their merit, referees should resolve such doubt at once by directing further collaborative study, looking toward either final adoption or deletion, as the findings dictate.

There are a few chapters in the "Methods of Analysis" which use the footnote device: "All methods are tentative unless otherwise designated." It is urged that this ambiguous device be abandoned. Each individual method should be plainly tagged; and incidentally, such a procedure would use up no additional space.

As to those methods designated "Tentative" (and those which now bear no designation), these seem to fall into the following broad groups:

(1) Those which could, and we feel should, be made official with or without further collaborative study, as the findings justify.

(2) Those which have been thought, we believe erroneously, to be, from their very nature, incapable of collaborative study.

(3) Those which are frankly for the purpose of making rough approximations rather than precise measurements.

(4) Those which are clearly obsolete.

A brief discussion of these four classes follows:

(1) No worthy method should be saddled with the term "Tentative," if by worthy we mean producing reliable results in capable hands. Any

method found unworthy of official adoption should, we believe, be dropped rather than continued as tentative; and referees should reach a decision as promply as possible. Pending such decision on methods not palpably unworthy or obsolete, the methods should be designated "Pending Official" or the equivalent. Such methods may be regarded as interim methods to be made official for inclusion in the revision of "Methods of Analysis" which follows their adoption. If found unworthy they should be dropped. It is not intended that the Book include methods in the "pending official" status.

(2) We believe that any method of analysis that is of proven usefulness is, almost by definition, capable of collaborative study. Take as an illustration the methods for the isolation of filth from foods in Chapter 42. These, we believe, could and should be studied collaboratively, not as methods of measurement which they are not, but rather as to whether they do or do not cleanly isolate the filth from the food itself. In such a study the collaborator would report a method as "satisfactory" if no food remained with the filth to obscure it. Satisfactory methods of isolation should then be adopted as official. We believe that other purely isolational methods could be handled in the same way.

(3) This class of methods serves the sole purpose of indicating to the analyst whether he needs to go through the longer and more accurate procedure, or whether he has no further regulatory or research interest in the sample. We believe that such methods should not be designated as either official or tentative, but rather called just what they are: "screening methods." Whatever the name, it should be uniformly used by all referees.

(4) The obsolete methods need little comment. The sooner they are deleted the better, for their omission will give the "Methods of Analysis" badly needed elbow room. Any doubt on the question should be promptly resolved by collaborative studies instigated by the referee.

By way of final comment, there is need for making a clear-cut distinction among (1) "Sampling Methods," which we feel should be rigidly restricted to the selection, from the "lot," of unit packages (or material, in the case of bulk shipments) for further attention; (2) "Drawing the Sample," which is concerned with exactly how the inspector breaks into the units selected, and withdraws, consolidates, and packs the sample so withdrawn; and (3) "Preparation of Sample," which describes just how the *analyst* treats it in order to make it homogeneous, before he weighs out the analytical charge. We recommend that referees adopt this plan of nomenclature, making any necessary changes in present headings.

A brief discussion of these three classes of sampling and subsampling follows:

(1) There are numerous treatises on the art of sampling in this re-

stricted sense of the word. It is enough to say that each individual task is always unique in some respect, and that therefore only the very broadest principles can be formulated for any given material. It is our view that all "methods" of this character should be deleted as at once too broad and (when "official") too restrictive.

(2) These, on the other hand, do have a place in the "Methods of Analysis," but we believe no such method should be retained unless the procedure has stood the test of collaboration, and then it should promptly be adopted as "official." Such collaboration needs to be wisely and carefully planned in advance, so that the experiment will, to the extent possible, isolate the sampling variations from the analytical ones. If, as seems improbable, a method is so abstract or abstruse as to make a collaboration impossible, it should be deleted.

(3) These methods are pretty well subject to the comment under (2), and little need be added here. It must be remembered that the choice is between a resort to some general language (such as "stir until homogeneous"), in which case the preparation is left entirely to the judgment of the analyst; and a set of directions for stirring, shaking, grinding, sieving, etc., which he must perforce follow. Which course to take in any specific case should be a matter of deep, and somewhat philosophical, reflection on the part of the responsible referee. The first course would call for no formal method of sample preparation at all; the second would call for a rigid "official" procedure.

We might point out certain headings that are somewhat difficult to classify, e.g. "Preliminary Examination," "Physical Examination," "Macroscopic Examination." In the main these deal with broad generalizations, directions, and the like. Perhaps referees should re-examine these as to their value, and then either delete them or give them some general heading, such as "General Suggestions" which would take them entirely outside the "Methods" classification.

In this connection, and also in connection with the above discussion on the entire process of sampling and sub-sampling, it might be wise to include a statement analogous to the first two parenthetical sentences in Chapter 1. These are: "(In view of the variability of soils, it seems impossible to devise an entirely satisfactory method for sampling. It is obvious that details of procedure should be determined by the purpose for which the sample is taken.)"

To sum up, your Committee makes the following recommendations to the Association:

To chart a course (including any necessary changes in the Constitution and By-laws) which, by the time the next edition of *Methods of Analysis* is issued, will have accomplished the following objectives:

(1) The complete deletion of all so-called "tentative" analytical and

sampling procedures which are palpably obsolete or unworthy.

(2) Adoption as "official" of such currently "tentative" procedures as are found worthy.

(3) The designation as "pending official" of those tentative methods not covered by (1), pending further study by the appropriate referee.

(4) Adoption as "official final action" of all those methods long designated as "official first action" which are found worthy of such adoption.

(5) Adoption of useful methods of the "screening" type, under a suitable heading which will not include either of the terms "tentative" or "official."

(6) The possible adoption of a heading, again without the words "official" or "tentative," to cover any useful suggestions as to physical, preliminary, or macroscopic examination, or as to handling and storage of the inspector's or analyst's sample.

W. B. WHITE, *Chairman*
K. D. JACOB
W. F. REINDOLLAR

REPORT OF THE SECRETARY-TREASURER

By HENRY A. LEPPER

The meeting of the executive committee was held on Sunday, October 19, 1947, at 10 a.m., in the Green Room of the Shoreham Hotel. All members were present and ex-president J. W. Sale served as the additional member in the absence of Dr. Ross. The audit of the firm of Snyder, Farr, and Company was presented and accepted.

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, INC.

BALANCE SHEET—SEPTEMBER 30, 1947

ASSETS

Current Assets:		
Cash, Lincoln National Bank	$23,091.40	
Office cash fund	61.12	$23,152.52
Accounts receivable	$ 4,038.42	
Less reserve for doubtful accounts	163.90	3,874.52
Accrued interest receivable, Government bonds		250.00
Inventories		5,057.87
Total Current Assets		$32,334.91
Investments		42,319.00
Total Assets		$74,653.91

LIABILITIES AND SURPLUS

Current Liabilities:		
Accounts payable		$ 2,245.16
Snrplus;		
Balance, October 1, 1946	$61,344.53	
Add net profit, year ended September 30, 1947,	11,064.22	
Balance, September 30, 1947		72,408.75
Total Liabilities and Surplus		$74,653.91

A vacancy on the Committee on Recommendations of Referees, due to the change in position of C. S. Ladd, no longer eligible to active membership, was filled by appointment of A. H. Robertson. The present chairman of the committee, W. F. Reindollar, was reappointed. The vacancy occurring on the editorial committee of *The Journal*, was filled by the appointment of J. B. Smith. Because of the practically complete sale of the 9,000 copies of the sixth edition of Methods of Analysis, the Secretary was instructed to negotiate the publication of an additional 1,000 or 2,000 copies, if the rate of sale indicates a sufficient demand therefore between now and the contemplated issue of the seventh edition in 1950. Authority for the purchase of an addressing machine and an adding machine for the business office was granted. The Secretary was instructed to arrange with other associations engaged in establishing methods of analysis involving the same topics as those of our Association a means if possible whereby uniformity in direction could be adopted by both, using the present harmonious relationship with the American Public Health Association as a guide.

Approved.

REPORT OF COMMITTEE ON NECROLOGY

ANDREW LINCOLN WINTON

Dr. Winton was born at Westport, Connecticut, January 26, 1864, and died October 17, 1946. After attending high school at Bridgeport, Connecticut, he graduated in 1884 from Sheffield Scientific School of Yale University with the degree of Ph.B. After graduation he became a member of the staff on the Connecticut Agricultural Experiment Station where he began his work on vegetable histology. Winton's first attendance at the Association Meetings was in 1891 at which time he presented a paper on the Determination of Potash. He was president of this Association in 1898. His interest in our Association continued to the end when only nine days before his death he wrote, "I have been looking ahead to the A.O.A.C. Meeting."

HENRY E. BARNARD

Dr. Barnard was born November 14, 1874, in Dumbarton, New Hampshire. He graduated from the University of New Hampshire in 1899. He received two honorary degrees, Ph.D. from Hanover College in 1913 and Sc.D. from New Hampshire in 1928. In 1901 he organized the New Hampshire State Laboratory of Hygiene and in 1905 performed the same duty for Indiana. He drafted the first food and drug law to be adopted after the passage of the Federal Law. He served as Federal Food Administrator during World War I and organized the Corn Industries Research Foundation and the National Farm Chermurgic Council during World War II, in both of which he was director. Dr. Barnard was principal Employment Specialist in Chemistry for National Personnel of Scientific and Specialized Personnel. He was active in the American Chemical Society, serving as a director from 1917 to 1920. He died December 31, 1946, in Indianapolis, Indiana, after an illness of five months.

ANDREW L. FELKER

Andrew L. Felker, New Hampshire's beloved Commissioner of Agriculture, died suddenly October 17, 1946, at the home of his son, Louis K. Felker at Laconia at the age of 77. He died at the end of a hard days work in the interest of his fellow farmers. He was born in Barrington July 6, 1869, and was appointed the first Commissioner of Agriculture for New Hampshire on January 10, 1914. At that time he had little help while today it requires 28 full time employees to handle the department. He organized and administered control and eradication of all diseases of live stock and poultry, laws controlling sale of seeds, feeds, fertilizers, insecticides and fungicides; dairy plant inspection; insect and plant disease disease control; apiary inspection and seed potato certification, and had the interest of all farmers in the State and was quick to establish laws for their benefit. He was the oldest commissioner of agriculture in the United States in years of continuous service, held the honorary degree of LL.D. and was active in many organizations. His place will be difficult to fill.

CHARLES ALBERT BROWNE

On February 3, 1947, Dr. Browne died in Washington, D.C., at the age of 76 years. His obituary published in the August 15, 1947, number of the *Journal* is an appreciation of his life and work and need not be repeated here. Suffice it to say that he achieved high rank in his chosen field, agricultural chemistry, particularly the chemistry of sugars, and withal was a charming scholar and gentleman. He was a warm supporter of this Association, serving as its president in 1925. He attended the meeting last fall and enjoyed to the full the contact with his many friends. In his passing we have all suffered loss.

WILLIAM HORACE ROSS

Dr. Ross was born on December 27, 1875 at River John, Nova Scotia, Canada, and died on May 16, 1947. His work on agricultural chemistry, started in Arizona in 1907 and continued after 1912 in the U. S. Department of Agriculture until his retirement in 1945, has been extremely fruitful. He has made many contributions to the development of methods for fertilizer analysis and has had a strong influence on the methods adopted by this Association. His work is more fully described in his obituary which appeared in the August 15, 1947, number of this *Journal*. The President's address, given at the October 1946 meeting of this Association, showed his broad understanding of the factors involving soil fertility and productivity. His counsel in the Association will be seriously missed.

BERNARD A. LINDEN

Born on December 17, 1893, in New York City, he died on March 9, 1947. Mr. Linden joined the staff of the Bureau of Chemistry in 1914 as a laboratory helper. He studied bacteriology and other biological sciences at Emerson Institute and George Washington University night schools and was promoted to the professional service in 1925. In his long career as a food bacteriologist he combined a high degree of technical skill and investigational ability in his work, notably on decomposition of fishery products, sanitation in food packing plants, and in the field of food poisoning. At the time of his death he was serving as supervising analyst in the Bacteriological Section, Division of Microbiology.

L. S. WALKER, *Chairman*
W. B. WHITE
E. L. GRIFFIN

REPORT OF THE COMMITTEE ON NOMINATIONS

President: G. H. Marsh, Director Chemistry Division, Montgomery, Ala.

Vice-President: L. S. Walker, Agricultural Experiment Station, Burlington, Vt.

Secretary-Treasurer Emeritus: W. W. Skinner, Kensington, Md.

Secretary-Treasurer: Henry A. Lepper, Food and Drug Administration, Washington, D. C.

Additional Members of the Executive Committee: W. A. Queen, Washington, D. C.; H. A. Halvorson, State Chemist, St. Paul, Minn.; W. B. White, Washington, D. C.; J. O. Clarke, Food and Drug Administration, Chicago, Ill.

W. H. MACINTIRE, *Chairman*
FRANK H. WILEY
LESLIE E. BOPST

Approved.

REPORT OF COMMITTEE ON RESOLUTIONS

Whereas, the officers of our Association have capably and efficiently performed their duties during the past year and have planned and carried through successfully this the 61st meeting at the close of the 63rd year of our organization, therefore, be it

Resolved, that we express our appreciation and thanks to President J. O. Clarke, Vice-president George H. Marsh, Secretary-Treasurer Henry A. Lepper, and other members of the Executive Committee, for unselfish and able service rendered.

Whereas, the progress of the work of this Association depends so much upon the effectiveness of the services of our Referees and Associate Referees, therefore, be it

Resolved, that the Association extend this expression of genuine appreciation to these workers who have given so generously of their time and efforts toward the accomplishment of the aims of this organization.

Whereas, the registration at the present meeting reveals the presence of representatives of State and Federal agencies and institutions in all parts of the Nation, and from Canada, therefore, be it

Resolved, that we hereby express our thanks to those agencies responsible for making possible the attendance of so many representatives; Be it further

Resolved, that the Secretary express to the management of The Shoreham Hotel the thanks of our Association for making available rooms and other facilities suitable for the successful conduct of the meeting.

GUY G. FRARY, *Chairman*
E. M. NELSON
J. J. TAYLOR

Approved.

CHANGES IN OFFICIAL AND TENTATIVE METHODS OF ANALYSIS MADE AT THE SIXTY-FIRST ANNUAL MEETING, OCTOBER 20, 21, AND 22, 1947*

The changes in the methods of the Association recorded below become effective March 17, 1948, as provided in section 8 of the by-laws, thirty days from the date of publication of this Report, Feb. 15, 1948.

1. SOILS

(1) The tentative method for fluorine, section **1.32** (p. 13), line 3, was changed by substituting "500" for "900."

2. FERTILIZERS

(1) The official method for sampling, section **2.1** (p. 20), was changed

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* Unless otherwise given all references in this report are to *Methods of Analysis, A.O.A.C.*, 1945.

by substituting "20" for the first "10" in line 7 and by addition of the following sentences between "bags" and "Thoroly" in line 7:

> "In sampling a bulk lot of fertilizer, draw not less than 20 cores from different regions of the lot. In sampling fertilizer in small containers (10 lbs or less) a single package may constitute a sample for the lot."

The whole section, as revised, was adopted as official, first action.

(2) The official volumetric method for phosphoric acid was changed, and the method as revised was adopted as official, first action, by—

(a) substituting "324.03" for "323.81" line 1 and "32.40" for "32.38" line 2, in section **2.10(b)**;

(b) deleting "and dilute to 200 ml with H_2O" from section **2.11** (p. 23) and designating the section as "**(a)**";

(c) by adding to section **2.11** the following:

> "**(b)** *Not applicable in preparation of solns by sulfuric acid digestion.*—Proceed as directed under **2.8 (a)**, **(b)**, or **(c)**, preferably **(a)** when these acids are a suitable solvent, to point where acid digestion of sample is completed. Add 25 ml of 10% $BaCl_2$ soln to the hot digestate, boil ca 2 min, and continue as directed under **2.8**."

(d) adding to section **2.12(a)** (p. 23) the following as the first sentence: "Prepare soln of sample as directed under **2.11(a)**."

(e) deleting the phrase, in section **2.12(b)** first line, "Not applicable to superphosphates and other fertilizers that contain sulfates (5)," and adding the following as the first sentence: "Prepare soln of sample as directed under **2.11(b)**."

(f) adding to section **2.12** a new paragraph, as follows:

> "**(c)** *Not applicable to superphosphates and other fertilizers that contain sulfate or to solns prepared with the aid of sulfuric acid.* (5).—Prepare soln of sample as directed under **2.11(a)**. Proceed as directed under **(b)**."

(3) The official method for potash was changed and the method as revised was adopted as official, first action, by substituting "95%" for "80%" in section **2.40(d)**, (p. 31), line 1, and section **2.42** (p. 32)' lines 12 and 15.

3. AGRICULTURAL LIMING MATERIALS

(1) The following method was adopted as tentative for neutralizing value of blast furnace slag.

Blast Furnace Slags.—Weigh 0.5 gram charge ground to pass 80-mesh sieve and transfer into a 250 ml Erlenmeyer flask. Wash down with small portions of water and introduce 35 ml of 0.5 *N* HCl while swirling. Heat to a gentle boil over Bunsen burner, *agitating the suspension continuously* until the bulk of the sample has dissolved. Maintain the boiling for 5 min.; cool to room temperature; then dilute with CO_2-free H_2O to about 150 ml and add 1 ml of 30% H_2O_2 and 3 drops of a 1% soln of Bromcresol green. (Methyl red indicator, 4 drops of a 2% soln, may be substituted for Bromcresol green with some advantage in visibility of end point. This indicator is very unstable, however, and requires renewal of buffer soln every few days.) Back titrate with 0.5 *N* NaOH, adding the first 15 ml rapidly and continue titration dropwise, contents of stoppered flask being agitated vigorously after each addition until the indicator tint matches or slightly oversteps that of a pH 5.2 phthalate buffer soln of like volume and indicator concentration after agitation for 2–3 seconds.

(2) The tentative procedures, section 3.3, p. 42, and the one above are to be annotated by the statement "without correction for sulphite content."

4. COSMETICS

The following method was adopted as official, first action:

GLYCEROL IN VANISHING CREAM

REAGENTS

Potassium periodate.—.02 *M*. Dissolve 4.6 grams of C. P. KIO_4 in ca 500 ml of hot water. Dilute to about 900 ml with water, cool to room temperature, and make to 1 liter.

Sodium hydroxide.—0.02 *N*. (See **43.5** or **43.6**)

Bromcresol purple indicator.—Dissolve 0.1 gm of indicator in 100 ml of alcohol.

Propylene glycol.—A pure product containing no free acidity or liberating acidic substances on oxidation is desirable. A product B.P. 85–86°C/10 mm is suitable.

DETERMINATION

Isolation of glycerol.—Place 2 to 10 gm of sample in a separatory funnel, add 25–50 ml of water, acidify slightly with dilute H_2SO_4 (10 gm/100 ml), and extract with successive portions of chloroform. Usually 4–5 portions of chloroform, each ca 35 ml, are sufficient to remove all chloroform soluble material. Wash the combined chloroform extract with 10 ml of water. Filter the aqueous solution and wash water through a cotton plug to remove droplets of chloroform, and receive filtrate in a 250 ml volumetric flask. Add 3 drops of bromcresol purple indicator to the filtrate and neutralize with carbonate free alkali (0.1 *N* NaOH is satisfactory) making the final adjustment with the .02 *N* NaOH. Dilute almost to the mark with water, and if necessary, add more alkali so as to maintain a light but definite purple color in the solution, and then complete the dilution to the mark.

Periodate oxidation.—Transfer an aliquot of the neutral soln preferably containing 30–40 mg of glycerol to a 100 ml volumetric flask and add 50 ml of .02 *M* KIO_4. Make to mark with water and allow to stand ca 1 hour. (Test for excess periodate which must be present in the oxidation mixture by adding $NaHCO_3$ and KI to a test portion; if excess is present, iodine will be liberated.)

Formic Acid Titration.—(Applicable in absence of substances yielding acid on periodate oxidation.) Transfer a 50 ml aliquot of the oxidized mixture to a titration flask, add 10 drops of propylene glycol (ca 0.5 ml), mix well, wash down the sides of the flask with water and allow to stand for 10 min. Add 3 drops of bromcresol purple indicator and titrate with 0.02 *N* NaOH to a light purple end point.

1 ml 0.02 *N* NaOH = 1.84 mg glycerol

5. ENZYMES

No additions, deletions, or other changes.

6. INSECTICIDES AND FUNGICIDES

(1) The following method was adopted as tentative.

TOTAL CHLORIDE IN EMULSIONS CONTAINING DDT, SOLVENT, EMULSIFYING AGENT, AND WATER

Weigh a quantity of well-mixed sample containing about 0.75 g of DDT in a tared weighing bottle. Wash into a 100-ml volumetric flask and make to volume with 99% isopropanol. Transfer a 10-ml aliquot to a 250–500 ml standard tapered Erlenmeyer flask. Place on a steam bath, and expel the isopropanol and water in a current of air. Remove last traces of solvent and water from the cooled flask. If drops of water still remain, add 10 ml of isopropyl alcohol and repeat the evaporation. Proceed as directed in method (6) *This Journal*, **30**, 65 (1947), line 2, beginning "Add 25 ml of 99% isopropanol. . . "

(2) The tentative method for DDT by total chlorine, *This Journal*, **30**, 64 (1947), was changed by deleting from the first "and," line 2, and ending with "and," line 6, and substituting therefor "add 10 ml thiophene-free benzene, dissolve the sample, make to volume with 99% isopropanol. Transfer a 25 ml aliquot to a 250–500 ml standard tapered Erlenmeyer flask. Add. . ."

(3) The tentative method for total chlorine in dusting mixtures containing DDT in absence of organic matter, *This Journal*, **30**, 64 (1947), was changed by deleting beginning with "From" line 3, p. 65, through "bath" line 4, and substituting therefor:

"Evaporate on steam bath until most of the benzene is removed. It is not desirable to evaporate to dryness, as DDT may decompose with loss of hydrochloric acid. Add 25 ml of 99% isopropanol and 2.5 g of metallic sodium in the form of ribbon or cut in small pieces and shake flask to mix sample with the alcohol. Proceed as in (1) *This Journal*, **30**, 64 (1947) beginning "Connect the flask."

(4) The tentative method for total chlorine in dusting mixtures containing DDT in presence of organic matter such as coloring matter, plant resins, etc., *This Journal*, **30**, 65 (1947), was changed by deleting beginning with "Proceed" line 7, through "water. . ." line 8, and substituting therefor:

"Evaporate on steam bath until most of the benzene is removed. It is not desirable to evaporate to dryness, as DDT may decompose with loss of hydrochloric acid. Add 25 ml of 99% isopropanol and add 2.5 g of metallic sodium in form of ribbon or cut in small pieces, and shake flask to mix the sample with the alcohol. Connect to a reflux condenser and boil gently for at least ½ hour. Shake flask occasionally. Eliminate excess metallic sodium by cautiously adding 10 ml of 50% isopropanol through the condenser at the rate of 1–2 drops per second. Boil for an additional 10 min., then add 60 ml of H_2O."

(5) The tentative method for total chlorine in mineral oil sprays containing DDT in the absence of organic matter (plant extractives material, organic thiocyanate) was changed by deletion beginning with "From," line 2, through "isopropanol," line 3, and substituting "Add 25 ml of 99% isopropanol and 2.5 g of metallic sodium and proceed as in (1) *This Journal*, **30**, p. 64 (1947), beginning "in the form of ribbon."

(6) The tentative method for p, p′ DDT, section **6.152** (p. 86), was changed by deleting beginning "Reflux" line 3, through the second "Add," line 4, and substituting therefor "Place in a water bath at 25° for 35–60 min, add 10 ml of H_2O and add . . ."

(7) The title of chapter 6 was changed to "Economic Poisons."

7. CAUSTIC POISONS

No additions, deletions, or other changes.

8. NAVAL STORES

No additions, deletions, or other changes.

9. GELATIN, DESSERT PREPARATIONS, AND MIXES

(1) The tentative method for jelly strength section **9.6** (p. 104), was deleted.

(2) The following method was adopted as tentative.

JELLY STRENGTH

"To 7.5 g of sample add from pipet, with stirring, 105 ml of water at 10° to 15°C. Let stand for one hour and then bring to 62° in 15 min. by placing in a water bath regulated at 65°; the sample may be swirled several times to aid solution. Finally mix by inversion, let stand 15 min. and place in a water bath at 10° controlled at ±0.1°. Chill for 17 hours. Determine jelly strength on Bloom Gelometer (2)* adjusted for a 4 mm depression and to deliver 200 gms shot/5 seconds (±5 gms), using the ½″ plummet."

10. LEATHERS

No additions, deletions, or other changes.

11. TANNING MATERIALS

No additions, deletions, or other changes.

12. PLANTS

No additions, deletions, or other changes.

13. BEVERAGES (NON-ALCOHOLIC) AND CONCENTRATES

No additions, deletions, or other changes.

14. MALT BEVERAGES, SIRUPS, AND EXTRACTS, AND BREWING MATERIALS

(1) The following details for alcohol by immersion refractometer, *Methods of Analysis*, 5th ed., Chapter XIV, section **5(c)**, were omitted from the 6th edition and should appear under section **14.5** (p.151) as **(c)**, and are as follows:

(c) *By immersion refractometer.*—Verify the percentage of alcohol as determined under (a) or (b) by ascertaining the immersion refractometer reading of the distillate and obtaining the corresponding percentage of alcohol from Tables **44.24** and **44.25**."

(2) The following official, first action (*This Journal*, 30, 67 (1947)) methods for malt were made official, final action:

Sampling, section **14.39** (p. 158).
Preparation of sample, section **14.40** (p. 159).
Bushel weight, section **14.41** (p. 159).
1,000 kernel weight, section **14.45** (p. 160).

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* Reference number refers to the selected references, Methods of Analysis, 6th Ed., p. 106.

Moisture in caramel malt and black malt, section **14.52** (p. 161).
Diastatic power, section **14.61** (p. 166).

(3) The following official, first action (*This Journal*, **30**, 67 (1947)) methods for beer were adopted as official, final action:

Extract of original wort, section **14.7** (p. 151).
Real degree of fermentation or real attentuation, section **14.8** (p. 152).
Apparent degree of fermentation or apparent attenuation, section **14.9** (p. 152).
Total acidity—Indicator Titration Method, section **14.10** (p. 152).
Total acidity—Potentiometric Titration Method, section **14.11** (p. 152).
H-ion concentration (*p*H)-Electrometric Method, section **14.12** (p. 153).

(4) The following official, first action (*This Journal*, **30**, 67 (1947)) methods for hops were adopted as official, final action:

Sampling, section **14.80** (p. 170).
Physical examination, section **14.81** (p. 170).
Preparation of sample for chemical analysis, section **14.82** (p. 171).
Moisture, section **14.83** (p. 172).
Resins, section **14.84–14.89**, inclusive (p. 172).

(5) The following official, first action (*This Journal*, **30**, 67 (1947)) methods for brewing sugars and sirups were adopted as official, final action:

Extract, section **14.90** (p. 174).
Non-extract (apparent water), section **14.91** (p. 174).
Fermentable extract (b) rapid fermentation method, section **14.92** (p. 174).
Protein, section **14.93** (p. 175).
Iodine reaction for unconverted starch, section **14.95** (p. 175).
Acidity, section **14.96** (p. 175).
H-ion concentration (*p*H), section **14.97** (p. 176).
Ash, section **14.98** (p. 176).
Total reducing sugars, Munson-Walker method, section **14.99** (p. 176).

(6) The following official, first action (*This Journal*, **30**, 67 (1947)) methods for wort were adopted as official, final action:

Specific gravity, section **14.103** (p. 176).
Original extract or original gravity, section **14.104** (p. 176).
Fermentable extract, section **14.105** (p. 176).
Iodine reaction, section **14.106** (p. 177).
Total acidity, section **14.107** (p. 177).
H-ion concentration (*p*H), section **14.108** (p. 177).
Color, section **14.109** (p. 177).
Protein, section **14.110** (p. 177).
Total reducing sugars, section **14.111** (p. 177).

(7) The official Milos test for caramel, **14.35** (p. 158) was deleted, first action.

15. WINES

(1) The official Milos test for caramel, section **15.38** (p. 188), was deleted, first action.

(2) The following method was adopted as official, first action:

CARAMEL

Mathers Test

Preliminary.—Before applying the following tests, the sample to be analyzed should be filtered through ordinary laboratory filter paper in order to remove suspended particles and sediment. The sediment or particles could also be eliminated by centrifuging. Use the clear portion for analysis.

Mather's Test.—To 10 ml of the above filtered wine or spirits previously introduced into a Babcock cream bottle or any convenient small centrifuge bottle, add 1 ml of pectin soln (made by dissolving 1 g of pectin in 75 ml of water and adding 25 ml of alcohol for preserving it. Shake well before using.) Add to the material to be tested in the centrifuge bottle about 3 drops of concentrated HCl and fill bottle with alcohol (ca 50 ml or more). Shake well and centrifuge for 5 min. or more and decant carefully the supernatant liquid off of the gelatinous residue. Dissolve the residue in the bottle by adding 10 ml water and shaking well. To this residue dissolved in water add about 3 drops of concentrated HCl and 50 ml or more of alcohol; shake well and again centrifuge. Repeat this process until the upper alcoholic layer is quite clear and colorless. After the final decantation of the water-white supernatent alcohol, the gelatinous residue is dissolved in 10 ml of hot water. A clear brown soln indicates caramel coloring. To further confirm caramel, add 1 ml of the following reagent (made by dissolving 1 g of 2,4-di-nitro-phenyl-hydrazine in 7.5 ml of conc. sulphuric acid and bringing the volume up to 75 ml with 95% ethyl alcohol; kept in a glass-stoppered bottle in which it will stay clear and stable for several months) to the residue dissolved in the 10 ml of hot water. Stand the bottle in a beaker of boiling water for 30 min. In the presence of substantial quantities of caramel a precipitate forms almost at once. Smaller amounts show as a precipitate before the 30 min. are up. Even the smallest amount of caramel will show a precipitate under the conditions described, but if the analysis has been conducted as described above, no precipitate will appear if caramel is absent. In order to be sure a precipitate has formed, pour the hot test soln from the bottle on a small filter paper and wash any residue with hot water. A reddish brown precipitate will be clearly seen on the filter. This precipitate, although amorphous, is quite characteristic and will always be the same if caramel is present. A low-power microscope affords a fine examination of the precipitate. If caramel is found to be present by the above test, check the sample as follows:

Confirmatory Test

Place 10 ml of wine or spirits in a small centrifuge bottle (Babcock cream bottle), approximately neutralize with 2% KOH soln, add 2 ml of 5% $ZnCl_2$ soln, and 2 ml of 2% KOH soln. Shake thoroughly and centrifuge for about 5 or more min. Decant the supernatent liquid carefully from the residue and add hot or boiling water. Shake thoroughly to wash precipitate, centrifuge, and again wash with hot water. Repeat this process until the upper aqueous water layer is quite colorless. Any amount of hot water may be used. To this well-washed residue add 50 ml or more of 85% alcohol containing 0.5% HCl. Shake well and centrifuge and decant off the upper liquid from any residue that remains below. If caramel is present a thin brown layer will be at the bottom of the centrifuge bottle. Add another portion of 85% alcohol containing 0.5% HCl, and again centrifuge. Repeat this process until the upper supernatant liquid is quite clear and colorless. To facilitate this washing of the caramel or any residue, the centrifuge bottle containing the 85% alcohol with 0.5% HCl may be dipped for a few min in the beaker of boiling water. After the final washing with acid alcohol and decanting off the alcoholic wash liquid, the residue, if any is present, is dissolved in 10 ml of water. A brown solution indicates caramel. To this brown soln, add 1 ml of the 2,4-di-nitro-phenyl-hydrazine reagent in the manner described above. If no precipitate appears after 30 min., no caramel is present. If the precipitate is difficult to see in the orange-colored test soln, pour it on the filter paper immediately after the 30 min boiling, and a 30 min cooling period is over.

(3) The directions of the tentative confirmatory tests for caramel, section **15.39** (p. 189) were changed by—

(a) Deleting the last sentence of paragraph 1 beginning "If there . . ." and paragraphs 2, 3, 4 and 5;

(b) substituting therefor the following—

To the brown solution in the Babcock or other test bottle add 1 ml of 2,4-dinitro-phenyl-hydrazine reagent and place bottle in beaker of boiling water and heat the bottle and its contents therein for 30 min, turn off the heat and allow the bottle to cool with the water for 30 min and examine; a brown precipitate confirms the presence of caramel.

16. DISTILLED LIQUORS

(1) The official modified Marsh test for caramel, section **16.39** (p. 302), was deleted, first action.

(2) The following test was adopted as tentative for distilled liquors and for cordials and liqueurs:

CARAMEL

Fulton Test

REAGENTS

(a) *Acetone reagent.*—Acetone, 500 ml; amyl alcohol, 200 ml; ethyl acetate, 200 ml; syrupy H_3PO_4, 50 ml; water, 50 ml.

(b) *Sodium di-hydrogen phosphate.*—NaH_2PO_4, 25 g; water, 100 ml.

DETERMINATION

To ca 5 ml of spirits add 10 ml of the acetone reagent and 3 ml of the NaH_2PO_4 soln, shake and let stand. Color in the lower layer indicates caramel.

(3) The official Milos test for caramel, section **16.41** (p. 202) was deleted, first action.

(4) The tentative method for alcohol by volume, section **16.46(b)** (p. 203), was changed by adding "except calibrate pycnometer at 15.56° and measure sample at that temperature."

(5) The Mathers test for caramel, see (2) and (3) under **15, Wines,** above, was adopted for distilled liquors and for cordials and liqueurs, as official, first action.

(6) The tentative method for caramel, section **16.60** (p. 204), was changed to drop reference to sections **16.39** and **16.41**.

17. BAKING POWDERS AND BAKING CHEMICALS

The official gasometric method for residual carbon dioxide, section **17.8** (p. 210), was deleted, first action.

(2) The following method was adopted as tentative:

RESIDUAL CARBON DIOXIDE IN BAKING POWDERS

GASOMETRIC METHOD

Place 1.7 g of baking powder in a clean, dry, 250 ml, wide-mouthed sohxlet extraction flask (flask A 17.5). Add 20 ml distilled water. Put flask *on the cover* of a water bath (single or multiple) in which boiling water is kept at a constant level of 2 inches below top of bath. The water in the bath should be boiling vigorously all through the determination and bath heated with gas or electricity. The opening in the cover of bath into which flask is set must be 3 inches in diameter. This prevents the flask from touching water, keeping it at a definite distance above. Evaporate contents of flask until there is no visible evidence of moisture in residue or inside surface of flask. If set-up is functioning properly, sample should be completely dry in 1½ to 2 hours. Leave the flask on water bath for 2 hours. Add 10 ml of distilled water and let stand until flask is at room temperature (ca 1 hour).

Determine carbon dioxide with Chittick apparatus as in 17.6, using correction factors in section 44.30. Shake the flask vigorously until further shaking produces no increment in the reading.

(3) The official method for available carbon dioxide, section 17.9 (p.210) was changed, first action, deleting "17.8 from 17.6" and substituting "the result from the tentative gasometric method from 17.6"

18. COFFEE AND TEA

No additions, deletions, or other changes.

19. CACAO BEAN AND ITS PRODUCTS

The following method was adopted as tentative.

TANNINS AND PIGMENTS

DETERMINATION

Place 25 g of sweetened products (such as milk chocolate, light sweet chocolate, skim, buttermilk, or malted milk chocolate) in a 250 ml centrifuge bottle. Extract three times with 100 ml of ether, shaking well, centrifuging, and decanting each time. Expel most of the residual ether by passing a moderate current of air through the bottle for ca 10–15 min. Add 40 ml of water, measured closely by graduate, to the bottle, stopper, and shake well for a minute or more to thoroughly disperse the contents of the bottle. Now add 40 ml of 1% sodium oxalate soln and again shake well. (If no milk solids are present, 80 ml of water can be used instead of the water and sodium oxalate.)

Place the bottle in the centrifuge and whirl for 10 min. or more at about 1800 r.p.m. Remove and decant the supernatant liquid into a 125 ml separatory funnel. Again add 20 ml of 0.5% sodium oxalate (equal volumes water and 1% Na oxalate) to the centrifuge bottle, shake well, and centrifuge as before.

Place 55 ml of absolute alcohol, 60 ml of acetone, and 130 ml of ethyl ether (all measured by graduate) in a 500 ml Erlenmeyer flask. Add also 28–30 g of salt (NaCl) and 1 ml of glacial acetic acid. Add the contents of the separatory funnel gradually in a small stream (requiring about 5 min time) to the contents of the flask while shaking the latter with vigorous rotation. Close the stopcock on the funnel and pour in the liquid of the second extraction from the centrifuge bottle. Shake funnel to rinse and add this extract also in a similar manner (shaking) to the contents of Erlenmeyer flask. Reserve the contents of the centrifuge bottle for further treatment. Stopper and shake the Erlenmeyer and if it contains a milk protein precipitate, pour the contents of the flask into centrifuge bottles, rinse flask with a wash of mixed ether, alcohol, and acetone in proportions used above. Centrifuge 10 min. and carefully decant the liquids into a 500 ml separatory funnel, retaining the precipitate in the bottles.

Rinse the bottles with about 30 ml of the wash of the three mixed solvents, pour out the contents of bottle on a Büchner funnel containing a Whatman No. 54 filter paper, and filter with suction. Add filtrate to contents of 500 ml separatory funnel. Draw off the lower layer and filter the top remaining layer through a plug of cotton into an 800 ml beaker. Wash funnel once with mixed solvent.

Measure 125 ml of absolute alcohol in a graduate. Add a portion of it to the bottle containing the residue reserved above. Stopper and shake well to disperse the residue and transfer it to a 300 ml Erlenmeyer flask, using all the alcohol. Add 2 ml conc. HCl to the flask, place on the steam bath, heat to the boiling point and 1 minute longer. Remove and filter on a 7-cm Büchner funnel using a CS&S No. 589 blue ribbon paper. Rinse the flask and wash the filter with 20 ml of absolute alcohol and with 25 ml of acetone.

Transfer the alcoholic filtrate quantitatively to the 800 ml beaker containing the previous extract. Evaporate the extracts on an electric hot plate to about 200 ml, add 75 ml of water, and continue boiling to about 75 ml. Add more water (40–50 ml) and continue boiling until all alcohol is driven off, adding water again if necessary. Transfer the liquid and any sediment quantitatively to a 250 ml beaker and boil down to ca 35 ml. Cool and pour the contents into a 50 ml graduate and note volume. Decant into a 200–250 ml flask and rinse beaker and graduate with small portions of water sufficient to make the total volume to 50 ml. Transfer any residue to the flask. Add 4 ml conc. HCl and 10 ml of 40% formaldehyde soln (Stiasny's reagent) to the contents of flask. Place the flask under a condenser and reflux for 30 min. Cool to room temperature and filter off the precipitate on a tared gooch crucible. Transfer the precipitate and wash with two 15 ml portions of wash soln (5 ml conc. HCl and 10 ml 40% formaldehyde added to each 100 ml H_2O). Then wash once with 10 ml of water. Dry the precipitate in the oven at 100°C., cool, and weigh. Obtain weight and per cent of precipitated tannins and pigments.

20. CEREAL FOODS

(1) The following methods were adopted as official, first action.

PHOSPHORUS IN CEREALS AND CEREAL PRODUCTS

REAGENTS

$Mg(NO_3)_2$ soln.—Dissolve 8 g MgO in HNO_3(1+1), avoiding excess of the acid; add a little MgO in excess, boil, filter from the excess MgO, Fe_2O_3, etc, and dilute to 100 ml.

Molybdate soln.—section **2.7 (a)**, p. 21.

DETERMINATION

(a) Transfer 1.0 g of sample to a size #3 porcelain casserole (140 ml capacity), add 3 ml $Mg(NO_3)_2$ soln, mix well, using a small glass rod, clean off the rod with a small piece of filter paper and place in the casserole. Drive off most of the moisture by drying in the oven at 100°C for about 2 hours, transfer to a cold muffle and ignite at 550°C until a white or gray ash is obtained (about 6–8 hours). Cool, cover with watch glass, take up with 10 ml HCl (1+4), add 5 ml conc. HCl, rinse off watch glass, and evaporate to dryness on steam bath, add 5 ml conc. HCl and 50 ml H_2O, heat 15 min on the steam bath, filter into 100 ml volumetric flask, cool and make to volume. Pipet 50 ml into 300 ml Erlenmeyer flask, neutralize to litmus with NH_4OH, make just faintly acid with HNO_3, dilute to 75–100 ml, add ca 15 g NH_4NO_3, and proceed under section **2.12**, p. 23, beginning "add sufficient molybdate soln to insure complete precipitation . . . " or

(b) Transfer 5.0 g of sample to a size No. 000 porcelain evaporating dish (35 ml capacity), mix well with 0.5 g Na_2CO_3, ash at 550°C until gray ash, cool, cover with watch glass, take up with 2 ml HCl (1+4), add 5 ml conc. HCl, rinse off watch glass, evaporate to dryness, add 5 ml conc. HCl and 10 ml H_2O and heat about 10 min on steam bath, filter into 100 ml volumetric flask, cool, make to volume. Pipet 10 ml aliquot in 300 ml Erlenmeyer flask and proceed as above beginning "neutralize to litmus with NH_4OH . . . " Report results as % P.

(2) The official dry ashing method for iron, sections **20.9–20.12** (pp. 238–239), was adopted as official, final action, for enriched macaroni products, degerminated, bolted, and whole corn meals.

(3) The wet ashing method for iron, *This Journal*, **30**, 71 (1947), was made official, final action.

(4) The tentative method for benzoyl peroxide bleach in flour, section **20.53** (p. 253), was deleted as applied to wheat flour but retained as applicable to rye flour and the title changed to Benzoic Acid in Rye Flour.

(5) The following method was adopted as tentative:

BENZOIC ACID IN WHEAT FLOUR

Place 50 g of flour in (preferably) glass-stoppered flask, add 30–40 glass beads (about 6 mm diam.), 0.1 g powdered iron, 100 ml ether or isopropyl ether. Allow to stand a few min. shake with a rotary motion and add slowly (preferably dropwise) 2.5 ml HCl from a Mohr pipet. Allow to stand overnight. Shake well with rotary motion, allow the flour to settle a few minutes, and decant through a Büchner funnel (100 mm) fitted with filter paper moistened with ether into a 500 ml suction flask. Add 50 ml ether, shake, and allow to settle a few minutes, decant as before, repeat twice more, transferring the whole contents to the funnel following the last addition. Transfer through a large funnel into a 250 ml separatory funnel, add 20 ml 5% $NaHCO_3$ soln, mix without too much vigorous shaking, and draw off the lower clear layer into a 125 ml Erlenmeyer flask, repeat with two more 20 ml portions of 5% $NaHCO_3$. Add to this soln 0.3 g decolorizing C (Nuchaw W), shake and filter (11 or 12.5 cm 589 white ribbon, S&S) into 125 ml Erlenmeyer flask, wash flask and filter with about 20–25 ml H_2O, add 2.0 ml H_2SO_4 (1+1) dropwise to avoid foaming out of the flask. (The soln should be definitely acid to litmus paper.) Transfer to a 125 ml separatory funnel, rinse out the flask with 12 ml ether and add to the funnel, shake gently with frequent release of pressure due to ether and CO_2. (During first extraction with ether, it is preferable to release pressure after each shake to avoid possibility of loss.) Repeat with two more 12 ml extractions with ether. Rinse out the flask each time with the ether. After each extraction draw off the aqueous soln into the same 125 ml Erlenmeyer flask and transfer the ether to a Pyrex test tube (about 50 ml capacity, 25 mm diam. and 150 ml length) and add 2 ml 10% NaOH. Hold top of tube firmly against the hand and shake vigorously, insert a piece of copper wire (1 mm diam. × 200 mm) into the tube, evaporate the ether very slowly on the steam bath. Remove copper wire. Place the tubes into a beaker of boiling water and evaporate nearly to dryness, add 0.5 ml 30% H_2O_2, followed by another 0.5 ml as soon as the foam condition permits. Continue evaporation until there is no apparent moisture in the tube. (The introduction of a gentle air blast into the tube hastens the evaporation.) Add from Mohr pipet 4 ml of (1+1) mixture of H_2SO_4 and fuming nitric acid, taking care to have it wash down the sides of the tube, heat

20 min. in a gently boiling water bath, immediately cool under tap, add 6 ml water while continuing to cool. Then slowly add 15 ml conc. NH_4OH with continuous shaking under the tap to keep soln cool. Add 2 ml of 6% hydroxylamine hydrochloride soln, stir, place in 65° water bath 5–6 min, stirring occasionally. Cool under tap, filter into similar tube and observe color of filtrate. Red or definitely pink color indicates the presence of benzoic acid. In case the color is not definite enough to indicate the presence of benzoic acid in a light bleach flour, repeat the analysis as above, except use 100 g flour, 200 ml ether, and 5 ml HCl.

***Quantitative determination.*—Transfer soln (within 30 min) to a 2 in. glass cell and read in neutral wedge photometer, using No. 51 filter or in other equally precise instrument at wave length 510 mμ. Standardize the instrument by placing in test tubes 0.4, 0.8, 1.0, and 1.2 mg benzoic in acetone soln (0.5 mg to 1 ml), adding 2 ml 10% NaOH, shaking to mix well and proceeding as above beginning "place the tubes into a beaker of boiling water." Report results in p.p.m. of benzoic acid.**

(6) The tentative method for soybean flour, sections **20.77–20.79**, and **20.82**, were changed to read as follows:

20.77 Moisture, "see **20.2** or **20.4**, with the exception that a 5 g sample be dried at 130° for 2 hours.

20.78 Ash, "see **27.9**."

29.79 Nitrogen, "Proceed as directed under **2.26** using 10 g K_2SO_4 or Na_2SO_4 and 0.7 g Hg or its equivalent in Hg with the option of using sodium alizarin sulfonate."

20.82 Oil or Petroleum Benzine Extract, "see **31.07** except that ca 2 g full fat soy flour or 5 g low defatted flour be extracted for 5 hours."

(7) The method for total solids in entire loaf of bread (raisin bread and bread containing raisins and fruits) section **20.84(b)**, p. 261, official, first action, *This Journal* **30**, 72 (1947), was adopted as official, final action.

21. COLORING MATTERS

(1) The following method was adopted as official, first action:

Intermediates

ANILINE

REAGENTS

Sodium nitrite soln.—Dissolve 79 g of $NaNO_2$ in 1 liter of water.

Sulfamic acid soln.—Dissolve 97 g of NH_2HSO_3 in 1 liter of water.

Coupling soln.—In a 500 ml wide-mouthed flask dissolve 0.1 g of 1-(4 sulfophenyl) 3-methyl-5-pyrazolone in 2 ml of ca 2.5 *N* NaOH soln and add 50 ml of H_2O and 10 grams of trisodium citrate. Cool to 10°C or below and hold at that temp. until used. This reagent must be freshly prepared for each determination.

DETERMINATION

Place 10 g of the dye, 200 ml of H_2O, and 5 g of trisodium citrate in a 500 ml round bottom flask equipped with a standard-taper neck. Connect the flask to a condenser with a suitable connecting tube. Distill 100 ml into a 100-ml graduated cylinder containing 1 ml of conc. HCl. Regulate the rate of distillation to require ca 1 hour.[1]

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[1] In the distillation of aniline from water insoluble colors, varying amounts of foaming and entrainment are encountered; in such cases, a trap may be used. The presence of a few particles of water insoluble color in the distillate, however, does not affect the results.

Place the distillate in a 500 ml Erlenmayer flask (washing out the graduate into the flask with a few ml of water), cool in ice to below 10°C and add 1 ml of the $NaNO_2$ soln. Mix well and let stand in the ice bath for 30 min. Then add 2 ml of the sulfamic acid soln, mix well, wash down the sides of the flask with a little H_2O cooled in ice. Let stand in ice 2 to 3 min, then pour into the flask containing the coupling soln.

Let stand at least one hour, heat on a steam bath for ½ hour, then heat to boiling and titrate the yellow color with 0.1 *N* $TiCl_3$. A 1% soln of FD&C Green No. 2 may be used as an indicator; in this case, a correction for indicator blank must be made. 1 ml of 0.1 *N* $TiCl_3$ = 0.0023 grams aniline.

(2) The tentative method for tartrazine, section **20.125** (p. 268), was changed by substituting "0.1 ml" for "1 ml" in 3rd line from end, and adopted as official, first action.

22. DAIRY PRODUCTS

(1) The following method was adopted as official, first action, for fluid milk and cream, cheddar type cheese, and soft uncured cheeses, and as tentative for other types of cheese, ice cream mix, sherbet mix, chocolate-flavored milks and skim milk, butter, sweet-cream and cultured buttermilks, fermented milks, goats' milk, cheese whey, and concentrated milks.

PHOSPHATASE TEST FOR PASTEURIZATION

REAGENTS

1. *Buffers:*

a. *Barium borate-hydroxide buffer.*—Dissolve 25.0 g of C. P. barium hydroxide [$Ba(OH)_2 \cdot 8H_2O$—fresh, not deteriorated] in distilled water and dilute to 500 ml. In another flask or cylinder dissolve 11.0 g of C. P. boric acid (H_3BO_3) and dilute to 500 ml. Warm each to 50°C, mix the two together, stir, cool to approximately 20°C, filter, and stopper the filtrate tightly (*p*H 10.6).

The buffer thus prepared is designated as the 25–11 buffer, the figures indicating the grams per liter of each of the respective reagents. Modifications in the quantities of these two reagents, necessary in preparing the appropriate buffers for testing various products, are indicated in Tables 1 and 2.

b. *Color development buffer.*—Dissolve 6.0 g of sodium metaborate ($NaBO_2$)[1] and 20 g of sodium chloride in water and dilute to 1 liter with water (*p*H 9.8).

c. *Color dilution buffer.*—Dilute 100 ml of color development buffer 1-b to 1 liter with water.

d. *Standard borax buffer,* 0.01-molar, for checking *p*H meter, *p*H 9.18 at 25°C.[2] Dissolve 0.9603 g of pure borax (Bureau of Standards Sample 187) in distilled water (distilled recently or freshly boiled and cooled) and dilute to 250 ml. Keep stoppered tightly.

2. *Buffer substrates:*

a. *For evaluating pasteurization.*—Dissolve 0.10 g of phenol-free crystalline disodium phenyl phosphate[3] in 100 ml. of the appropriate (Tables 1 and 2) barium borate-hydroxide buffer 1-a.

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[1] Obtainable from Amend Drug and Chemical Company, Inc., 117 East 24th Street, New York 10 N.Y.

[2] All *p*H values reported herein were determined at 25° C or corrected to that temperature.

[3] Obtainable, relatively pure, from Applied Research Institute, 2 East 23rd Street, New York 10, N.Y.

b. *For quantitative results with raw milk and raw-milk products.*—Dissolve 0.20 g of the phenol-free crystalline disodium phenyl phosphate in 100 ml of the appropriate (Tables 1 and 2) barium borate-hydroxide buffer **1-a**.

3. *Protein precipitants:*

a. *Zinc-copper precipitant for milk.* Dissolve 3.0 g of zinc sulfate ($ZnSO_4 \cdot 7H_2O$) and 0.6 g of copper sulfate ($CuSO_4 \cdot 5H_2O$) in water and dilute to 100 ml with water. The precipitant thus prepared is designated as the 3.0–0.6 precipitant.

b. *Zinc-copper precipitant for unripened cheese.* Dissolve 6.0 g of zinc sulfate and 0.1 g of copper sulfate in water and dilute to 100 ml with water. This precipitant is designated as the 6.0–0.1 precipitant.

c. *Zinc precipitant for ripened cheese and for butter.* Dissolve 6.0 g of zinc sulfate in water and dilute to 100 ml with water. This precipitant is designated as the 6.0 precipitant.

The quantities of the respective reagents to use in preparing the precipitants for testing other products, not mentioned under "Protein precipitants" above, are indicated in Tables 1 and 2.

4. *BQC* or *2,6-dibromoquinonechloroimine solution* (*Gibbs' reagent*)—Dissolve 40 mg of BQC[4] powder in 10 ml of absolute ethyl or methyl alcohol and transfer to a dark-colored dropper bottle. This reagent remains stable for at least a month if kept in the ice tray of a refrigerator. Do not use it after it begins to turn brown.

5. *Other reagents:*

a. *Copper sulfate, 0.05 percent, for standards.*—Dissolve 0.05 g of copper sulfate in water and dilute to 100 ml.

b. *Butyl alcohol.*—Specify n-butyl alcohol, boiling point 116–118°C. To adjust the *p*H, mix 50 ml of the color development buffer **1-b** with a liter of the alcohol.

6. *Phenol standards:*

a. *Stock soln.*—Weigh accurately 1.0 g of pure phenol, transfer to a liter volumetric flask, dilute to a liter with water, and mix. One ml contains 1 mg of phenol. Use this stock soln to prepare standard solns. It is stable for several months in the refrigerator.

b. *Preparation of standards.*—Dilute 10.0 ml of the stock soln **6-a** to a liter with water, and mix. One ml contains 10 micrograms (0.00001 g; 10 mmg, or 10 units) of phenol. Use this standard soln to prepare more dilute standard solns; *e.g.*, dilute 5, 10, 30, and 50 ml to 100 ml with water to prepare standard solns containing 0.5, 1.0, 3.0, and 5.0 mmg or units of phenol per ml, respectively. Keep standard solns in the refrigerator.

In a similar manner, prepare from the stock soln as many more concentrated standard solns as may be needed, containing, for example, 20, 30, and 40 units per ml.

Measure appropriate quantities of the phenol standard solns into a series of tubes (preferably graduated at 5.0 and 10.0 ml) to provide a suitable range of standards as needed, containing 0 (control or blank), 0.5, 1.0, 3.0, 5.0, 10.0, etc., to 30 or 40 units. To increase the brightness of the blue color and improve the stability of the standards, add 1.0 ml of 0.05% copper sulfate soln **5-a** to each.

Add 5.0 ml of color dilution buffer **1-c** and add water to bring the volume to 10.0 ml. Add 4 drops (0.08 ml) of BQC[4], mix, and allow to develop for 30 min. at room temperature. If the butyl alcohol extraction method is to be used in the test, extract the standards as described under "Conducting the test."

Read the color intensities with a photometer, subtract the value of the blank from the value of each phenol standard, and prepare a standard curve (straight line). When the standards are to be used for visual comparisons they should be stored in a refrigerator.

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[4] Obtainable from Applied Research Institute.

TABLE 1.—*Phosphatase test modifications for different kinds of cheese and cheese of different ages*

KIND OF CHEESE	AGE OR EXTENT OF CURING; OTHER DETAILS	BUFFER FOR OPT. pH (9.85–10.20)	PRECIPITANT	CRITERION, EXPERIMENTAL, PHENOL EQUIVALENT[a]
				mmg /0.25 g.
Cheddar, granular, stirred curd, hard cheese	<1 wk.	25-11[b]	6.0-0.1[c]	3
	1 wk.–1.5 mo.	25-11	6.0[d]	3
	1.5–4 mo.	26-11	6.0	3
	>4 mo.	27-11	6.0	3
Washed curd, soaked curd, Colby	<1 wk.	25-11	6.0-0.1	3
	1 wk.–2 mo.	25-11	6.0	3
	>2 mo.	26-11	6.0	3
Swiss, Gruyère	<1 wk.	25-11	6.0-0.1	3
	1 wk.–1 mo.	25-11	6.0	3
	1–3 mo.	26-11	6.0	3
	>3 mo.	27-11	6.0	3
Brick, Muenster	<1 wk.	25-11	6.0-0.1	3
	1 wk.–1 mo.	25-11	6.0	3
	1–2 mo.	25-11	6.0	3
	>2 mo.	26-11	6.0	3
Edam, Gouda	<1 wk.	25-11	6.0-0.1	3
	1 wk.–2 mo.	25-11	6.0	3
	2–4 mo.	26-11	6.0	3
	>4 mo.	27-11	6.0	3
Blue mold, blue	<1 wk.	25-11	6.0-0.1	3
	1 wk.–1 mo.	26-11	6.0	3
	1–4.5 mo.	27-11	6.0	3
	>4.5 mo.	28-11	6.0	3
Camembert, Limburger	<1 wk.	25-11	6.0-0.1	4
	1 wk.–1 mo.	25-11	6.0	4
	1–2 mo.	26-11	6.0	4
	>2 mo.	27-11	6.0	4
Monterey	<1 wk.	25-11	6.0-0.1	3
	1 wk.–2 mo.	25-11	6.0	3
	>2 mo.	26-11	6.0	3
High moisture Jack	<1 wk.	25-11	6.0-0.1	3
	1 wk.–2.5 mo.	25-11	6.0	3
	>2.5 mo.	26-11	6.0	3
Provolone, pasta filata	<1 wk.	25-11	6.0-0.1	3
	1 wk.–1 mo.	25-11	6.0	3
	1–3 mo.	26-11	6.0	3
	>3 mo.	27-11	6.0	3

TABLE 1.—(*continued*)

KIND OF CHEESE	AGE OR EXTENT OF CURING; OTHER DETAILS	BUFFER FOR OPT. pH (9.85–10.20)	PRECIPITANT	CRITERION, EXPERIMENTAL, PHENOL EQUIVALENT[a]
				mmg /0.25 g.
Parmesan, reggiano, monte, modena, Romano, asiago old	<1 wk.	25-11	6.0-0.1	3
	1 wk.–2 mo.	26-11	6.0	3
	2–6 mo.	27-11	6.0	3
	6 mo.–1 yr.	28-11	6.0	3
	>1 yr.	29-11	6.0	3
Asiago fresh	Same as Cheddar			
Asiago medium	<1 wk.	25-11	6.0-0.1	3
	1 wk.–1 mo.	25-11	6.0	3
	1–3 mo.	26-11	6.0	3
	>3 mo.	27-11	6.0	3
Gorgonzola	Same as blue			
Cottage,[e] cook cheese, koch kaese	Dry	25-11	6.0-0.1	1
	Moist	25-11 (8+2)[f]	4.5-0.1	1
Cream cheese		25-11 (7+3)	4.5-0.1	3
Semi-soft cheese	<1 wk.	25-11	6.0-0.1	3
	1 wk.–1 mo.	25-11	6.0	3
	>1 mo.	26-11	6.0	3
Soft ripened cheese	<1 wk.	25-11	6.0-0.1	4
	1 wk.–1 mo.	25-11	6.0	4
	>1 mo.	26-11	6.0	4
Nokkelost, kuminost, sage cheese	<1 wk.	25-11	6.0-0.1	3
	1 wk.–1.5 mo.	25-11	6.0	3
	1.5–4 mo.	26-11	6.0	3
	>4 mo.	27-11	6.0	3
Pasteurized process, past. proc. pimiento, past. proc. with fruits, meats, etc.	Soft, mild	25-11	6.0	3
	Medium firm	26-11	6.0	3
	Firm, sharp (incl. Swiss, Gruyère)	27-11	6.0	3
Past. proc. cheese foods; past. proc. cheese foods with fruits, meats, etc.	Same as pasteurized process			
Past. proc. cheese spreads; past. proc. cheese spreads with fruits, meats, etc.	Soft, high moisture, incl. cream spreads	25-11	6.0	3
	Less soft, incl. Blue	26-11	6.0	3

TABLE 1.—(*continued*)

KIND OF CHEESE	AGE OR EXTENT OF CURING; OTHER DETAILS	BUFFER FOR OPT. pH (9.85–10.20)	PRECIPITANT	CRITERION, EXPERIMENTAL, PHENOL EQUIVALENT[a]
				mmg/0.25 g.
Cold pack, club; cold pack cheese foods; cold pack cheese foods with fruits, meats, etc.	Mild to medium flavored, soft	26-11	6.0	3
	Sharp, firm	27-11	6.0	3

[a] Values higher than those shown indicate under-pasteurization.
[b] Grams $Ba(OH)_2 \cdot 8H_2O$ and H_3BO_3 respectively, per liter.
[c] Grams $ZnSO_4 \cdot 7H_2O$ and $CuSO_4 \cdot 5H_2O$ respectively, per 100 ml.
[d] Grams $ZnSO_4 \cdot 7H_2O$ per 100 ml.
[e] See also more sensitive modification in text (p. 90), alternative.
[f] Eight parts of 25-11 buffer plus 2 parts of water.

TABLE 2.—*Phosphatase test modifications for various dairy products other than cheese*

PRODUCT	QUANTITY OF SAMPLE	BUFFER FOR OPTIMUM pH (9.85–10.20)	PRECIPITANT	CRITERION, EXPERIMENTAL, PHENOL EQUIVALENT[a]
Milk:				*mmg*
Fresh	1 ml.	25-11[b] (5+5)[c]	3.0-0.6[d]	2/0.5 ml.
Old or slightly sour	1 ml.	25-11	6.0[e]	2/0.5 ml.
Cream:				
Fresh	1 ml. or 1 g.	25-11 (5+5)	3.0-0.6	2/0.5 ml. or 0.5 g.
Old or slightly sour	1 ml. or 1 g.	25-11 (8+2)	4.5	2/0.5 ml. or 0.5 g.
Ice cream mix	1 ml.	25-11 (8+2)	4.5-0.1	2/0.5 ml.
Sherbet mix	1 ml.	25-11 (5+5)	3.0-0.6	2/0.5 ml.
Chocolate drink	1 ml.	25-11 (8+2)	4.5-0.1	2/0.5 ml.
Butter	1 g.	18-8	6.0	2/0.5 g.
Sweet buttermilk	1 ml.	25-11 (5+5)	3.0-0.6	2/0.5 ml.
Cultured buttermilk and fermented drinks:				
Medium acid	1 ml.	25-11	6.0	2/0.5 ml.
Very acid, $pH<4.5$	1 ml.	26-11	6.0	2/0.5 ml.
Goats' milk[f]	3 ml.	27-11	7.5-0.1	1/1.5 ml.
Cheese whey	1 ml.	25-11 (5+5)	3.0-0.6	2/0.5 ml.

[a] Values higher than those shown indicate under-pasteurization.
[b] Grams $Ba(OH)_2 \cdot 8H_2O$ and H_3BO_3, respectively, per liter.
[c] Five parts of 25-11 buffer plus 5 parts of water.
[d] Grams $ZnSO_4 \cdot 7H_2O$ and $CuSO_4 \cdot 5H_2O$, respectively, per 100 ml.
[e] Grams $ZnSO_4 \cdot 7H_2O$ per 100 ml.
[f] Four-hour incubation period; use 7.0 ml. of filtrate and add 3.0 ml. of color development buffer 1-b.

PHOTOMETRIC DETERMINATION

To read the color in aqueous soln, use a filter with maximum light transmission in the region of 610 mμ wave length.

To read the color in butyl alcohol, extract the color as described above and centrifuge the sample for 5 min. to break the emulsion and to remove the moisture suspended in the alcohol layer. A Babcock centrifuge can be adapted for this purpose by making special tube holders as follows: Slice a section ½ inch thick from a rubber stopper of suitable diameter to fit into the bottom of the centrifuge cup. Glue together two cork stoppers of appropriate diameter, bore through the center a hole of proper size to hold the tube snugly, and insert the double cork section into the cup. After centrifuging, remove nearly all of the butyl alcohol by means of a pipet with a rubber bulb on the top end. Filter the alcohol into the photometer cell and read with a filter with maximum light transmission in the region of 650 mμ wavelength.

If more than approximately 4 ml of butyl alcohol is required for the photometer used, conduct the test in a larger tube and extract the color, in both the test and the standards, with the necessary quantity of butyl alcohol rather than with 5 ml specified above.

SAMPLING

1. Milk and other fluid products.—Mix the product well, pour several ml into a small tube, stopper the tube, and keep it in a refrigerator.

2. Hard cheese: Take a sample from the interior with a *clean* Roquefort trier, place in a small tube, stopper the tube, and keep it in a refrigerator.

3. Soft and semi-soft ripened cheese.—Harden the cheese by chilling it in the freezing chamber of a refrigerator. Taking special precautions to avoid contaminating the sample with phosphatase that may be present on the surface, use either of the following methods for sampling:

(a) Cut a portion from the end of the loaf or from the side of the cheese, extending in at least 2 inches if possible or to a point somewhat beyond the center in the case of a small cheese. Cut a slit ½ to ¼ inch deep at least halfway around the portion and midway between the top and bottom. Break the portion into two parts, pulling it apart so that it breaks on a line with the slit, being careful not to contaminate the freshly exposed, broken surface. Remove the sample from the freshly exposed surface at or near the center of the cheese.

(b) Remove the surface of the area to be sampled—*e.g.*, the end and the adjacent sides—with a clean knife or spatula, to a depth of ½ inch. Clean the instrument and hands with hot water and phenol-free soap and wipe them dry. Remove the freshly exposed surface to a similar or greater depth, and repeat the cleaning. Then take the sample from the center of the freshly exposed area, preferably at or near the center of the cheese in the case of a small cheese.

4. Process cheese, spreads, butter, and other non-fluid products.—Take the sample from beneath the surface with a clean knife or spatula.

5. Ice cream and sherbet.—Melt the portion removed and allow it to remain melted for an hour or longer before testing, testing it as a fluid product.

Avoid the use of samples contaminated with mold.

6. Preservation.—If a preservative is necessary, for liquid products add 1 to 3 per cent of chloroform; for solid products put 1 to 3 ml of chloroform in the container, cover with a plug of cotton, insert sample and stopper container tightly. *Label preserved samples*: "*Poison, preservative added.*"

CONDUCTING THE TEST

The chemical principles involved in the detection and measurement of milk phosphatase activity are the same for all dairy products. Some modifications, described below, have been found necessary for different dairy products, because of their differences in physical properties, compositions, and especially buffering capacities.

Cheese:

Step 1. Weigh, on a *clean* balance pan or watch glass, a 0.50-g sample (preferably in duplicate) and place in a culture tube 16 or 18×150 mm. Similarly, weigh another sample and place in a tube as a control or blank. If the cheese is sticky, weigh the sample on a piece of wax paper about 1×1 inch and insert the paper with the sample into the tube. Macerate the blank and the test with a glass rod about 8×180 mm.

Step 2. Add *to the blank* 1.0 ml of the appropriate (Table 1) barium buffer **1-a** (without substrate added), macerate with the rod, leave the rod in the tube, heat for about a minute to at least 85°C (185°F) in a beaker of boiling water with the beaker covered so that the entire tube is heated to approximately 85°C, cool to room temperature, and macerate again with the rod.

Step 3. Add *to the test* 1.0 ml of barium buffer substrate **2-a** or **2-b** (Table 1) and macerate.

From this point, treat the blank and the test in a similar manner.

Add 9.0 ml of the appropriate barium buffer substrate **2-a** or **2-b** (total, 10.0 ml added), and mix. The rod may be left in the tube during incubation; if it is removed at this point, cut a piece of filter paper ca 1×1 inch, wrap and hold it tightly around the rod, rotate the rod while withdrawing it from within the tube so as to wipe the rod clean, insert the paper with the adhering fat into the tube, and stopper the tube.

Step 4. Incubate in a water bath at 37–38°C for 1 hour, mixing or shaking the contents occasionally.

Step 5. Place in a beaker of boiling water for nearly a minute, heating to approximately 85°C (use a thermometer in another tube containing the same volume of liquid), and cool to room temperature.

Step 6. Pipet in 1.0 ml of the zinc precipitant **3-c** for ripened cheese, or the zinc-copper precipitant **3-b** for unripened cheese, and mix thoroughly (*p*H of mixture, 9.0–9.1).

Step 7. Filter (5-cm funnel, 9-cm Whatman No. 42 or No. 2 paper recommended), and collect 5.0 ml of filtrate in a tube, preferably graduated at 5.0 and 10.0 ml.

Step 8. Add 5.0 ml of color development buffer **1-b** (*p*H of mixture, 9.3–9.4).

Step 9. Add 4 drops[6] of BQC, mix, and allow the color to develop for 30 min at room temperature.

Step 10. Determine the intensity of blue color by either of two methods:

a. *With a photometer:* Read the color intensity of the blank and that of the test, subtract the reading of the blank from that of the test, and convert the result into phenol equivalents by reference to the standard curve described under "Phenol standards." The butyl alcohol extraction method ordinarily is unnecessary when using a photometer.

b. *With visual standards:* For quantitative results in borderline instances, *e.g.*, tests yielding 0.5 to 5 units of color, extract with butyl alcohol **5-b**. Add 5.0 ml of the alcohol and invert the tube slowly several times, centrifuge if necessary to increase the clearness of the alcohol layer, and compare the blue color with the colors of standards in the alcohol.

With samples yielding more than 5 units, compare the colors in aqueous tests with those of aqueous standards.

Step 11. *Dilution method for quantitative results:* In tests that are observed to be strongly positive during color development—*e.g.*, 20 units or more—in which 4 drops of BQC may be much less than sufficient to combine with all of the phenol, pipet an appropriate proportion of the contents into another tube, make up to 10.0 ml with color dilution buffer 1-c, and add 2 drops more of BQC in the case of an unripened product or 4 drops in the case of a ripened product. With each test, dilute and treat the blank in the corresponding manner. Dilute each strongly positive test thus until the final color is within the range of the visual standards or photometer. Allow 30 min. for color development after the last addition of BQC, and make the reading at the end of the 30-min. period. To correct, multiply by 2 for a 5+5 dilution, by 10 for a 1+9 dilution, and by 50 for a 1+9 followed by a 2+8 dilution.

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[6] For merely detecting under-pasteurization in testing unripened cheese, 2 drops are sufficient, provided the visual standards likewise are prepared with 2 drops.

Alternatively, to reduce the amount of yellow off color, add 2 instead of 4 drops of BQC after each dilution, and allow the color to develop. Then test the completeness of color development by adding a third drop. Repeat the dilution procedure until the addition of an extra drop does not cause any further increase in the amount of blue color.

Step 12. *Calculation and evaluation of result:* When using 0.5 g of solid sample and adding a total of 11.0 ml of liquid, multiply the value of the reading by 1.1 to convert it to units of color or phenol equivalents per 0.25 g of cheese. The result, if desired, may be converted to phenol equivalents per 1 g. by multiplying by 4.4. Evaluate the result by comparing it with the criteria of pasteurization in Table 1.

Milk and other fluid products:

Step 1. Pipet a 1.0-ml sample (preferably in duplicate) into a tube and pipet 1.0 ml into another tube as a control or blank. In testing cream, the sample may be weighed (1.0 g) if desired; in testing goats' milk, pipet in a 3.0 ml sample (Table 2).

Step 2. Heat the *blank* to the temperature indicated under "Cheese: Step 2," and cool to room temperature. From this point, treat the blank and the test in a similar manner.

Step 3. Add 10.0 ml of barium buffer substrate 2-a or 2-b (Table 2), stopper the tube, and mix.

Steps 4 to 11, inclusive. Follow the directions given for the corresponding steps under "Cheese" above, substituting the appropriate precipitant (Table 2) in Step 6, and, for merely detecting under-pasteurization, using 2 rather than 4 drops of BQC in Step 9.

Step 12. *Calculation and evaluation of result:* When using 1.0 ml of fluid sample and adding 11.0 ml of liquid (total liquid 12.0 ml, 5.0 ml of filtrate used), multiply the value of the reading by 1.2 to convert it to phenol equivalents per 0.5 ml of sample. If desired, the result may be converted to phenol equivalents per 1 ml by multiplying by 2.4. Evaluate the result by comparing it with the criteria of pasteurization in Table 2.

Butter:

Step 1. Weigh, on a piece of wax paper about 1×1 inch on a balance, a 1.0-g sample (preferably in duplicate) and insert the paper with the sample into the tube. Similarly, weigh another sample and place in a tube as a control or blank.

Step 2. Heat the *blank* to the temperature indicated under "Cheese: Step 2." and cool to room temperature. From this point, treat the blank and the test in a similar manner.

Step 3. Add 10.0 ml of barium buffer substrate 2-a or 2-b (prepared with 18–8 barium buffer, Table 2), stopper the tube, and mix.

Steps 4 to 11, inclusive. Follow the directions given for the corresponding steps under "Cheese" above, mixing the contents frequently and thoroughly during incubation, substituting the appropriate zinc precipitant (Table 2) in Step 6, and, for merely detecting under-pasteurization, using 2 rather than 4 drops of BQC in Step 9.

Step 12. *Calculation and evaluation of result:* When using 1.0 g of butter and adding 11.0 ml of liquid, multiply the value of the reading by 1.1 to convert the result to phenol equivalents per 0.5 g of butter. Evaluate the result by comparing it with the criterion of pasteurization in Table 2.

MODIFICATIONS FOR DIFFERENT CHEESES AND OTHER DAIRY PRODUCTS

Different dairy products, and different kinds of cheese and cheeses of different ages, have different buffering capacities, and therefore some of them require modification of concentrations of the reagents. The modifications of the barium buffer needed to produce optimal *p*H conditions during incubation (9.85–10.20), and of the precipitant to yield uniformly clear filtrates and to minimize interference during color development under optimal *p*H conditions (9.3–9.4), are specified in Tables 1 and 2.

With some samples, especially cheese samples of unknown history, slight deviations from the optimal *p*H range may occur, but such deviations do not very materially affect the results. For example, *p*H values as low as 9.6 or as high as 10.35 during incubation have been found to result in an average decrease of not more than 20 per cent below the maximum in the quantity of phenol liberated. The use of the 25–11 buffer substrate with samples for which the 27–11 buffer substrate is specified yields *p*H values not lower than 9.8.

A trace of cloudiness in the filtrate, following the use of the precipitant as prescribed, indicates that the concentration of barium hydroxide in the buffer was not sufficiently great, *i.e.*, that the buffer substrate was not sufficiently alkaline. For example, the 25–11 buffer diluted 5+5 with water, for use with fresh milk, may yield a cloudy filtrate if used with old milk having a *p*H below approximately 6.0, or with milk that has soured, and the test should be repeated with a more concentrated buffer and precipitant. Likewise, the 25–11 buffer, for use with unripened cheese, may yield a cloudy filtrate if used with ripened cheese, indicating that the concentration of the buffer used was not sufficient. Increasing the concentration of zinc sulfate in the precipitant also eliminates turbidity of the filtrate.

In testing cheese of unknown history or age, information as to the percentage of solids, especially the nonfat solids, is useful as an indication of the correct buffer to use; cheese with a relatively high percentage of nonfat solids generally requires the use of a relatively concentrated buffer to adjust the *p*H of the mixture correctly.

For precise quantitative results on unknown samples, adjust the *p*H to 10.0–10.05 for the incubation.

Cottage cheese curd is heated in the presence of considerable acid during manufacture, and therefore its phosphatase values are comparatively low. Alternatively, to increase the sensitivity of the test on cottage cheese, apply the following modifications: Use a 1.0-g sample, 27–11 buffer substrate, 2-hour incubation, 6.0–0.1 precipitant, and a pasteurization criterion of 2 units per 0.5 g.

Phosphatase activity is much less in goats' milk than in cows' milk. The details of a modification designed to increase the sensitivity of the test applied to goats' milk are specified in Table 2.

To test concentrated milk products, reconstitute the product with water to its original concentration of milk solids and test in the manner specified for the original product.

To test for the presence of microbial phosphatase, which is indicated by blue color in the blank prepared as directed above, repeat the determination, adding 1 ml of the appropriate barium buffer (without substrate) to the blank and heat it for

5 min. in boiling water in a covered beaker. If the blank treated thus is negative, it indicates that the blue color in the original blank was due to microbial phosphatase, *i.e.*, a "false positive" sample.

PRECAUTIONS

The length of time that the crystalline disodium phenyl phosphate and the BQC powder will remain stable can be increased greatly by keeping them in the freezing chamber of a refrigerator.

The glassware, stoppers, and sampling tools should be scrupulously clean, and it is desirable to soak them in hot, running water after cleaning.

The bottles containing solid barium hydroxide and the barium buffer must be kept stoppered tightly to prevent absorption of carbon dioxide.

Phenolic contamination from plastic closures on reagent bottles has been encountered, and therefore the use of plastic closures should be avoided. Rubber stoppers should not be used in flasks in which butyl alcohol is stored. Glass or cork stoppers should be used.

(2) The tentative methods for phosphatase test for pasteurization, sections **22.43–22.57**, were deleted.

(3) The following was adopted as tentative as a rapid screening method.

MOISTURE IN CHEESE

Weigh 2–3 g of prepared sample into moisture dishes with tight fitting covers. Partially dry on the steam bath with lids removed and then insert in a forced draft oven which has come to equilibrium at 130 ± 1°C. Dry for 1.25 hours (with covers entirely off), cover tightly, remove from oven, cool, and weigh.

(4) The following method was adopted as tentative.

BUTTER

Preparation of Sample—Mechanical Stirrer Method

EQUIPMENT

(a). A cylindrical jar holder mounted on the shaft of a hand centrifuge or similar apparatus for rotation of the jar on a vertical axis by means of a crank and transmission mechanism (See Fig. 1). The sample container must be cylindrical.

(b). A common steel spatula with wooden handle and fairly stiff blade at least 1¼″ wide by 5″ long with blade shaped to fit the jar as indicated in Fig. 2.

PREPARATION

Warm the sample in the closed sample jar to a temperature of about 25°C, avoiding liquefaction of any portion to the extent that oily and aqueous phases separate. The most easily managed samples are those that have been warmed to room temperature overnight, but it is not essential that the entire contents of the jar be thus uniformly tempered, provided all parts are at least in a plastic state. In principle the sample should be warmed only enough to permit initiation of mixing.

Place the jar in the jar-holder and wedge it in position with shims of corrugated pasteboard. Remove the cover and scrape off any butter adhering thereto with the back of the spatula blade. Immerse the blade in the jar with the edge against the side, holding it at a slight angle from the vertical and the plane of the blade at a slight angle to the radius. Operate the crank with the other hand, slowly at first, increasing speed as lumpiness decreases. In a short time the lumpiness disappears rather abruptly, and from this point on tilt the spatula occasionally on its horizontal

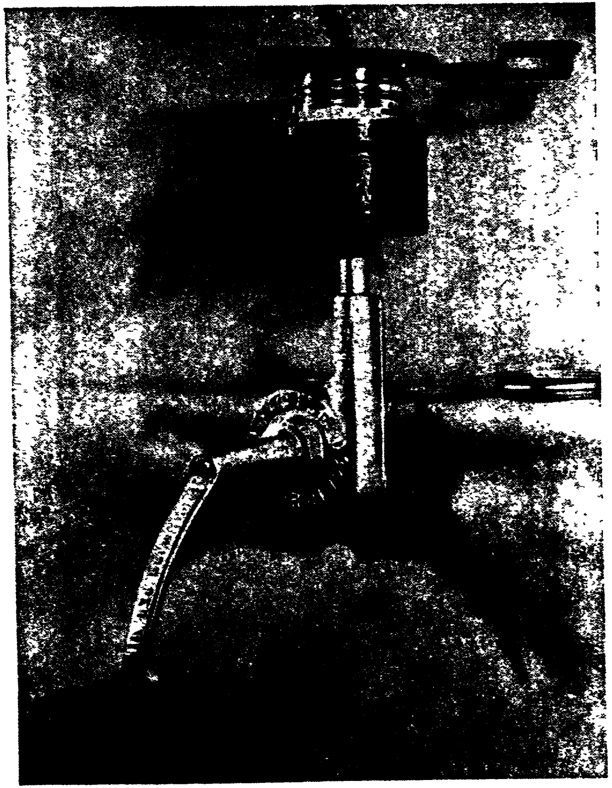

Fig. 1.—Mechanical stirrer for preparation of butter samples showing special blade for rapid mixing.

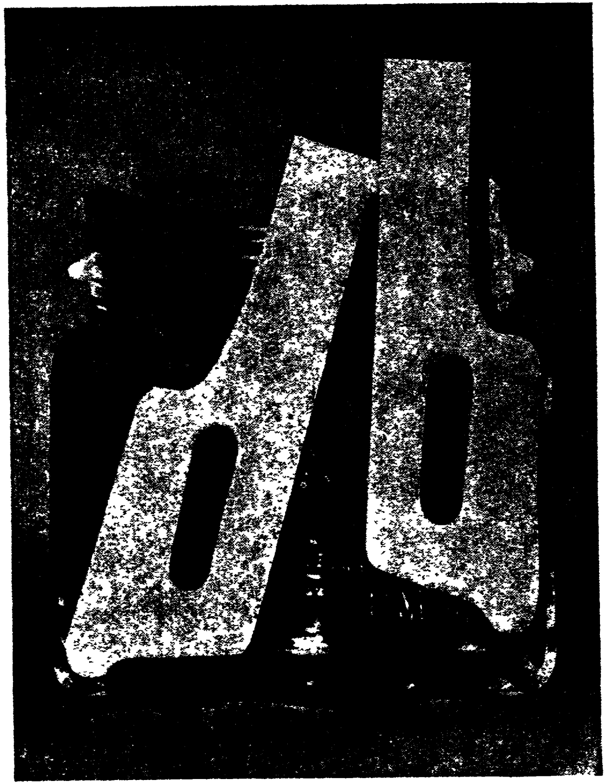

Fig. 2.—Special blade in two positions facilitating mixing action.

axis as illustrated in Fig. 2, move it back and forth to bring the toe of the blade a little past the axis of rotation, then up and down against the side of the jar, so twisted on its vertical axis as to throw the material first to the side and then to the center of the jar. After lumpiness disappears a mixing time of one minute is normally adequate. When mixing has been completed wipe off the blade against the mouth of the jar and replace the cover promptly. Start the analysis without undue delay—in no case later than one hour after mixing.

(5) The following editorial change was made in the method for fat in ice cream and frozen desserts, section **22.149** (p. 343), line 4.

After "60" add "for 20 min with occasional shaking" and add "cool and" before "Proceed."

(6) The following editorial change was made in the official method for added water in milk:

Section **22.28(a)** (p.311), line 8, after "suspected" add "Scale readings are identical on Bausch & Lomb refractometers except those bearing serial numbers 4000 to 10000, in which case readings of 38.6 and 39.6 correspond, respectively to 39 and 40 on the Zeiss instrument (Zeiss scale = B&L scale times 1.0092)."

Section **22.29(a)** (p.312), line 3 after "H_2O" add "Scale readings are identical on Bausch & Lomb immersion refractometers except those bearing serial numbers 4000 to 10000 in which case a reading of 37.9 corresponds to 38.3 on the Zeiss instrument."

Section **22.30** (p.312), line 5 after "H_2O" add "Scale readings are identical on Bausch & Lomb immersion refractometers except those bearing serial numbers 4000 to 10000, in which case a reading of 35.6 corresponds to 36 on the Zeiss instrument."

(7) The following editorial changes were adopted in section **22.33** (p. 314) of the cyroscopic method for added water in milk.

Insert "depression" after "point" line 6, p. 316.

Delete "depression" in line 9 and in 3rd line from end of method, p. 316.

(8) The sediment test, sections **22.40–22.42**, was dropped.

(9) The following method from "Standard Methods of Milk Analysis," American Public Health Association, was adopted as tentative.

SEDIMENT IN FLUID MILK

A. COLLECTION OF SAMPLE

(a) *Mixed Sample Method.*—Use 1 pt. samples. A retail pint bottle is satisfactory. Thoroly mix the milk in bulk containers before removal of the test portion. Where quart or other sizes are involved, including 10 gal. cans, transfer with a small strainer all floating extraneous matter, such as flies, hairs, large chunks of dirt, etc., if any are present, to the mounted disc. B (g). Avoid contamination of the milk with dirty stirrers or by any other means.

(b) *Off-bottom Method.*[1]—Use 1 pt. samples but do not disturb the bulk milk except as needed to insert the tester carefully to the bottom of the container and remove therefrom the 1 pt. portion, C (b). Where quart, or other sizes, are involved, including 10 gal. cans, transfer with a small strainer all floating extraneous matter, such as flies, hairs, large chunks of dirt, etc., if any are present, to the mounted disc, B (g). Avoid contamination of the milk with dirty stirrers or by any other means.

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[1] J. Amer. Public Health Assn., 37, 728 (1947).

B. APPARATUS AND MATERIALS

The tester should be of simple construction, easily cleaned and quickly adjustable between samples so as to permit the sanitary removal of the used disc and the replacement therein of the unused disc. Before use, check the tester for duplicability of results, B (e). Milk must not by-pass the filter disc.

(a) *Sediment testers for mixed sample method.*—Pressure gravity or vacuum types may be used.

(b) *Sediment testers for off-bottom method.*—Single unit type for intake of 1 pt. of milk on the up stroke of the plunger and discharge through the disc on the down stroke, or, two-unit type, one for removal of 1 pt. of milk from the bottom of the can and the other to filter the sample. Sampling device for the off-bottom method must be designed to permit insertion to the bottom of the milk can. Milk enters the tester through an opening measuring 1 in. in diameter.

(c) *Cotton sediment discs.*[2]—Provide special lintine cotton discs, diameter 1¼ in., for use over a flat wire screen in the tester so as to expose a filtration area at the center measuring 1⅛ in. in diameter. Testers and discs usually can be purchased from dealers in dairy supplies.

(d) *Preparation of standard sediment discs.*—Make a uniform mixture of oven dried (100°.) materials which meet the following screening specifications:

	Per cent
Cow manure, 40 mesh	53
Cow manure, 20 mesh, retained on 40 mesh	2
Garden soil, 40 mesh	27
Charcoal, 40 mesh	14
Charcoal, 20 mesh, retained on 40 mesh	4

Moisten 2 gm of the above mixture with 4 ml of 1 % aerosol soln or other suitable wetting agent, and 46 ml of 0.75% gum soln such as gum arabic, and then make up to 100 ml with a 50% by weight sucrose soln. Mix thoroly, transfer a 10 ml portion (200 mg of standard sediment) with a large tip, graduated pipette to a flask and make up to a liter with 50% by weight sucrose soln. When thoroly mixed, each ml contains 0.2 mg of sediment. Add definite volumes of the sediment mixture to ¾ pt. of filtered milk. Mix thoroly and pass the mixture thru a standard sediment disc with a filtering area measuring 1⅛ in. in diameter. Wash the container promptly with ¼ pt. of filtered milk. For a permanent record, mount and spray the disc with 40% formaldehyde or with a soln containing 2.5 g menthol and 2.5 g thymol made up to 100 ml with alcohol. Following the above method, prepare a series of discs containing the sediment remaining from 0.0, 0.2, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 12.0, and 14.0 mg of the standard mixture. Identify each disc on the permanent record with the amount of sediment in milligrams used to prepare the pad.

(For the purpose of comparison, the entire series of discs may be used. It will usually be found more convenient to select a few discs denoting the variations in grade which are applicable to the particular investigation being made. Where grading charts are prepared and where reports are made, the chart and report must indicate whether the mixed sample or off-bottom method has been used. If standards are to be handled or to be used for any appreciable length of time, place them under glass, transparent plastic sheets, or other suitable materials. In using standards, the sediment discs of the sample tested should be graded to the nearest standard sediment disc whether the actual amount of sediment is above or below the standard. Gross pieces of material, such as whole flies, large chunks of manure, etc., should be disregarded in grading the pads, but should be plainly listed in connection with each pad on which they occur.)

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[2] J. Milk Tech., 7, 260 (1944).

(e) *Checking sediment testers.*—Because of mechanical variations in sediment testing devices, check each tester frequently for duplicability of results as directed below.

To check the sediment testing devices, proceed as follows: Measure the actual amount of milk delivered to make sure that 1 pt. is withdrawn and passes thru the disc. Thoroly mix 2 gm of the oven-dried mixture described in B (d) with 4 ml of 1% wetting agent soln and 46 ml of 0.75% gum soln, such as gum arabic, and then make up to 100 ml with a 50% sucrose soln. Mix thoroly and transfer 10 ml with a large tip, graduated pipette to 10 gal of clean filtered water in a clean milk can. After thoroly agitating the mixture, remove a 1 pt. portion with a clean pint measuring device and filter the pint mixture, water, and sediment, thru a 1¼ in. diameter area of a standard lintine disc mounted in a Büchner, or other suitable funnel, of proper size. After thoro agitation again, remove a pt. sample with the sediment testing device from the same can and pass it thru the lintine disc in exactly the same manner as used in milk testing. This procedure is to be followed for testers used for mixed sample and off-bottom methods. Repeat this operation several times with the tester to determine whether or not all discs obtained in this manner are comparable with the disc obtained by filtering thru a Büchner funnel as described above.

(f) *Photograph of sediment disc standards.*—Photographic standards may be used as a guide in grading sediment pads. Preferably, use actual discs as directed in B (d). A photograph showing the complete range, as there described, is obtainable at $1.50 each from the American Public Health Association, 1790 Broadway, New York 19, N. Y. Do not use photographs which have become faded, stained, soiled, or otherwise damaged.

(g) *Use of discs.*—Discs may be mounted on special size paper or placed individually in transparent waxed envelopes. Place the disc on the paper or in the envelope while still moist with milk. As the milk dries, it serves as an adhesive. To prevent decomposition, observe spraying treatments in B (d). Spray the discs with a 40% formaldehyde soln or with a soln containing 2.5 gm menthol and 2.5 gm thymol, made up to 100 ml with 95% alcohol. Do not use glue to affix the disc to the paper. If the discs become detached, moisten with a few drops of water and remount. Protect discs from contamination.

C. DIRECTIONS

(a) *Mixed Samples.*[1]—Pass a 1 pt. sample, taken from the well mixed milk, thru a properly adjusted, firm cotton lintine disc held in correct position in the sediment tester. Milk varies considerably in its rate of flow through the lintine discs. Pasteurized milk may be more difficult to filter than raw milk. Other factors influencing the rate of flow are the temperature, fat content, the degree of clumping of the fat globules, stage of lactation, the presence of mastitic milk, and the amount of sediment in the sample.

Remove disc from tester, mount and identify. Grade by comparison with standard discs as provided in B (d). Always indicate on report whether pad is graded when wet or when dry. The character of the sediment may be determined by microscopic examinations.

(b) *Off-bottom Samples.*—For either type of off-bottom tester, the pt. sample is taken not more than ½ in. off the bottom of the unstirred can of milk while the sampling device is moved diametrically across the bottom of the can. Take the sample on the up stroke of the plunger, synchronizing the withdrawal of a full pint with the movement of the head of the tester once completely across the bottom of the can. To do this, push the head of the tester away as the plunger is pulled up in the tube to suck in the pint sample. Proceed as directed in C (a) after "Remove disc from tester. . . ."

23. EGGS AND EGG PRODUCTS

No additions, deletions, or other changes.

24. FISH AND OTHER MARINE PRODUCTS

The following method was adopted as tentative.

INDOLE IN SHRIMP, OYSTERS, AND CRABMEAT

APPARATUS AND REAGENTS

Color Reagent.—Dissolve 0.4 g p-dimethylaminobenzaldehyde in 5 ml acetic acid and mix with 0.92 ml phosphoric acid and 3 ml HCl. The purity of the p-dimethylaminobenzaldehyde exerts a strong influence on the intensity of the reagent blank. The yellow commercial reagent must be purified as follows:

Dissolve 100 g p-dimethylaminobenzaldehyde in 600 ml dil. HCl (1+6). Add 300 ml water and precipitate the aldehyde by slowly adding 10% NaOH soln with vigorous stirring. As soon as the precipitated aldehyde appears white, stop the addition of the NaOH, filter and discard the precipitate. Continue the neutralization until practically all of the aldehyde has been precipitated. The neutralization should not be carried to completion since the last 4–5 g of aldehyde are inclined to be colored. Filter and wash the precipitate with water until the washings are no longer acid. Dry the aldehyde, which should be practically white, in a desiccator.

Acetic Acid.—C. P. If this reagent gives a pink color with the color reagent, purify by distilling 500 ml in all glass still with 25 g $KMnO_4$ and 20 ml H_2SO_4.

Chloroform.—U.S.P.

Sodium Sulfate.—Saturated aqueous solution.

Hydrochloric Acid.—Dilute 5 ml conc. HCl to 100 ml with water.

Standard Indole Soln.—Weigh accurately 20 mg indole into a 200 ml vol. flask and dilute to mark with alcohol. Keep stock solution under refrigeration and do not use after two weeks.

Distillation Apparatus.—Use a separate steam generator for each unit. Steam generator may be made from 1 liter Erlenmeyer flask and connected to all-glass steam distillation apparatus with minimum use of rubber tubing. Distillation flask (capacity not less than 500 ml) is connected to straight bore condenser through a spray trap. A 500 ml Erlenmeyer flask is an effective receiver. Foil covered rubber stoppers may be used in absence of all-glass apparatus. (Unprotected natural or synthetic rubber connections and stoppers cause variable distillation blanks.)

Note: Guard against traces of chlorine in the distilled water since they may partly or entirely inhibit the development of the indole color.

PREPARATION OF SAMPLE

Crabmeat:

Weigh 25 g or 50 g of drained crabmeat (depending upon the amount of indole expected) into a Waring blendor, add 80 ml water and mix for several min. until a smooth homogeneous mixture is obtained. Transfer quantitatively to the distillation flask, using a minimum of wash water to rinse the mixing chamber.

Oysters:

Weigh 50 g of drained oyster meats into a Waring blendor, add 80 ml water and mix until a smooth homogeneous mixture is obtained. Transfer quantitatively to the distillation flask, using a minimum of wash water to rinse the mixing chamber.

Shrimp:

Weigh 25 g or 50 g of peeled raw or cooked shrimp (depending upon the amount of indole expected) into a Waring blendor, add 80 ml of alcohol and mix for several min. until the shrimp are finely divided, and transfer quantitatively the mixture to the distillation flask, using a minimum quantity of alcohol to rinse the mixing chamber.

DETERMINATION

Connect flask to steam distillation apparatus and gently apply steam. Care should be exercised until the distillation is well started in order to avoid excessive foaming which is encountered occasionally. Apply sufficient heat to the distillation flask to maintain 80–90 ml volume. Collect 350 ml distillate in ca 45 min. If alcohol was used in the preparation of the sample, collect 450 ml of distillate. Wash the condenser with a small amount of alcohol and allow to drain into receiving flask containing the distillate.

Transfer the distillate to a 500 ml separatory funnel, add 5 ml dilute HCl and 5 ml sodium sulfate soln. Extract successively with 25, 20, and 15 ml portions of chloroform. Shake each portion vigorously for at least one min. Combine and wash the 25 and 20 ml chloroform extracts in a 500 ml separatory funnel with 400 ml water, 5 ml sodium sulfate soln and 5 ml dil. HCl. Save wash water. Filter the combined extracts through a plug of cotton into a dry 125 ml separatory funnel. Wash the 15 ml portion, using the same wash water and combine with the other portions in the 125 ml separatory funnel.

Add 10 ml of the color reagent to the combined extracts, shake vigorously for exactly two min. and allow acid layer to separate as completely as possible. Drain until 9.0 ml have been secured.

Fill to 50 ml mark with acetic acid, mix well, transfer the soln to a suitable photometer cell and measure color photometrically at 560 mμ. The color soln may be diluted with acetic acid, containing 9.0 ml color reagent per 50 ml of soln, provided blanks are determined at the same dilutions.

Prepare a standard curve by the above procedure by steam distilling a series of freshly prepared diluted solutions of standard indole soln. Determine the distillation blank in a like manner, omitting addition of indole.

25. FLAVORING EXTRACTS

(1) The official method for alcohol, section 25.2 (p.365), was changed by deleting from "or" to "25.9" and substituting "but measure the sample used at 15.56° in a pycnometer (Fig. 1 or Fig. 2, p. 192–3) calibrated at that temperature."

(2) The tentative method for alcohol in ginger extract, section 25.6 (p. 376), was changed to read "Proceed as directed in 16.6(b)."

26. FRUIT AND FRUIT PRODUCTS

No additions, deletions, or other changes.

27. GRAINS AND STOCK FEEDS

(1) The following editorial change was adopted in section **27.59** (p. 419): Insert "Cool, make to measured volume of 50 or 100 ml and mix" before the last sentence beginning "Compare" line 11.

(2) The following method was adopted as tentative:

CALCIUM AND PHOSPHORUS

PREPARATION OF SAMPLE

(a) Proceed as directed under **12.5** to end of paragraph.

(b) Weigh a sample of suitable size (2 grams) into a Kjeldahl flask (500 or 300 ml). Add 20–30 ml of concentrated nitric acid and boil gently until all of the easily oxidizable matter is oxidized (30–45 minutes). Cool the soln somewhat and add 10 ml of perchloric acid (70–72%). Boil very gently, adjusting the flame until the soln is water-white or nearly so, and dense white fumes appear. Use particular care not to boil to dryness at any stage in the procedure. Cool slightly, add 50 ml of distilled water, and boil to drive out any remaining nitrogen fumes. Cool, dilute, filter, and make to volume in a 250 ml flask.

DETERMINATION

Calcium.—With soln prepared as above, proceed as directed in section **27.47** beginning "Pipet 25 ml . . . ," line 4.

Phosphorus.—With soln prepared as above, proceed as directed in section **2.12(a)**.

(3) The following method was adopted as tentative:

FAT IN FISH MEAL

Weigh 4–5 g of the meal to the nearest 0.01 g into an alundum or paper extraction thimble, cover with light layer of cotton, and extract in a continuous extractor for 16 hours with acetone.

At the end of this extraction period, distill off acetone until volume in flask is 10–15 ml. Transfer this oil soln to a 100 ml tared beaker washing flask free of all oil with fresh acetone and evaporate with a current of warm air. A convenient method is to place flask on a grill (*e.g.*, over a steam radiator) in front of a small electric fan.

When no moisture or acetone can be observed, place beaker in a vacuum oven at 80°C and apply a vacuum of 24 to 25 inches for 1 hour. Transfer to desiccator, cool, and weigh.

Transfer the extracted meal residue from the thimble to a 150 ml beaker. Remove any remaining solvent by heating on warm grid and then add 60 ml of 4 *N* HCl. Digest for one hour at or near the boiling point on a hot plate, occasionally stirring with a glass rod, adding water as necessary to maintain volume in the beaker. (Thoro removal of all acetone is necessary preliminary to this digestion, for otherwise vaporization of solvent will carry meal particles over side of vessel on to hot plate.) Filter through a 12.5 cm fluted filter. Wash residue on filter until free of acid, using methyl red indicator on portions of filtrate to follow progress of washing. Place filter and meal in a 150 ml beaker and dry in air oven at 80–90°C for one hour. Transfer filter paper and contents to a thimble and extract 16 hours with acetone. Remove solvent and weigh extract as above. The sum of the weights of extracts represents total fat.

(4) The following editorial changes were made in the official method, sections **27.28–27.30**, for crude fiber, p. 408:

27.28

(a) Delete "Contains" substitute ".255 *N*."

(b) Delete "Contains" substitute ".312 *N*."

(c) Add to paragraph:

"Gooch grade, medium fiber, acid washed and ignited is usually satisfactory but should be tested for chemical stability and filtering speed before use."

27.29

(c) Delete "appreciable," line 1. Add "Retention can be tested by running filtrate thru Gooch" after "rapid," line 2.

27.30

Lines 8 and 9—delete "ca every 5 min. in order to mix charge thoroly" and substitute "frequently until sample is thoroly wetted."

(5) The official method for crude protein, section **27.10** (p. 405), was changed to read "Determine N as directed under **2.24**, **2.25**, or **2.26**. With products containing 30% of protein or over, proceed as directed under **2.26**. Multiply results by 6.25."

28. MEATS AND MEAT PRODUCTS

No additions, deletions, or other changes.

29. METALS, OTHER ELEMENTS, AND RESIDUES IN FOODS

No additions, deletions, or other changes.

30. NUTS AND NUT PRODUCTS

No additions, deletions, or other changes.

31. OILS, FATS, AND WAXES

(1) The S.P.A. method for unsaponifiable residue, section **31.40** (p. 504) was made official, final action.

(2) The F.A.C. method for unsaponifiable residue, sections **31.37–31.39** (p. 504) was deleted, final action.

(3) The method for squalene, sections **31.41–31.43** (p. 505), was adopted as official, final action.

32. PRESERVATIVES AND ARTIFICIAL SWEETENERS

(1) The following method was adopted as tentative:

VOLATILE FATTY ACIDS IN BAKERY PRODUCTS

PREPARATION OF SOLUTION

(a) *Air-Dried Bread.*—For analysis of the air-dried product prepare sample as directed in section **20.83**.

(b) *Fresh Bread or Cake.*—For analysis of the fresh product or for analysis of cake, which is often difficult to air-dry without spoilage, pass the sample through a meat grinder, equipped with a ⅛" hole plate, and reduce to a finely divided condition by rubbing through an 8 mesh sieve. Proceed with the analysis promptly.

DETERMINATION

Transfer 25 g of air-dried or 35 g of fresh sample to a 250 ml volumetric flask. Add 100 ml of H_2O and mix by swirling until all particles are wet and any lumps are completely broken up. Add 25 ml of ca normal H_2SO_4, shake for 2 min and let stand for ½ hour, shaking occasionally to stir up the particles. Do not allow bread to clog in the neck of flask. This can be prevented by avoiding too vigorous shaking. Wash down with a small amount of water if necessary. Add 15 ml of 20% W/V phosphotungstic acid, shake for 2 min. and make to volume. Transfer mixture to centrifuge bottle and centrifuge for 10 min., at 1000–1500 r.p.m. Disregard turbidity. Decant (should be about 180 ml) and pipet 150 ml to a 500 ml flask equipped with water condenser for refluxing. Add ca 1.0 gm Ag_2SO_4 and heat for 5 min. after boiling begins. Cool flask under running water to room temperature—reflux connected—wash down condenser, transfer contents of flask to a 200 ml vol flask and make to volume. Mix in a 400 ml beaker with 3–5 gm of filter cell, stir and filter (S.S. #589 15 cm preferred). Pour back to give best possible clearness. Difficulty in obtaining a clear filtrate indicates insufficient Ag_2SO_4. Test for excess Ag by allowing a few drops of filtrate to flow into a test tube containing about 5 ml of 5% NaCl in 1+3 HNO_3. If excess of Ag is not indicated, add more Ag_2SO_4 (0.2 gm), shake and filter on a new paper.

Transfer 150 ml of the chloride-free filtrate to the standard distillation flask and proceed as directed under section **24.11.**

Collect only the two initial portions of distillates (50 ml and 200 ml) unless the ratio of their titers is less than the standard C ratio for propionic acid by more than 0.1. In this case collect two additional 200 ml portions of distillate for calculation of acids higher than propionic.

Determine formic acid in composite of distillates and correct the titrations for the titer contributed by this acid. Using the prescribed aliquots, the results in terms of mg of acid per 100 grams of sample is calculated from the determined ml of 0.01 *N* acid in the distillation by means of the following factors: Formic acid—4.09; acetic acid—5.33; propionic acid—6.58.

(2) The following method was adopted as tentative:

THIOUREA IN ORANGE JUICE

REAGENTS

(a) *Grote's reagent.*—Dissolve 0.5 g of sodium nitroprusside in 10 ml of H_2O in a 50 ml Erlenmeyer flask. Weigh out 0.50 g of hydroxylamine hydrochloride ($NH_2OH \cdot HCl$) and 1 g of $NaHCO_3$. Add the $NH_2OH \cdot HCl$ to the nitroprusside soln, give it a single whirl, then add immediately the 1 g of $NaHCO_3$ with no agitation. Allow the reaction to proceed without any molestation until the evolution of CO_2 subsides to only a small evolution. Then swirl to dissolve any remaining $NaHCO_3$. When the evolution of CO_2 practically ceases, add 0.10 ml (11 small drops) of bromine. A second evolution of gas occurs. When agitation no longer produces effervescence make to 25 ml volume with distilled water and filter. Test the reagent for its efficacy as follows: Dilute 2 ml as in (b), add 1 ml diluted reagent to 10 ml solution composed of 5 ml reagent (d), 5 ml H_2O, and 1 drop acetic acid. A strong blue color should develop in 5 min. If it does not, new reagent (a) should be prepared and the test repeated. Allow to stand at room temperature for 5–10 hours to age the solution. The soln should be a mahogany brown color. If it is of a greenish cast, it is not as effective a reagent and soon loses its value. Preserve this stock soln in the refrigerator and it will keep for several weeks.

(b) *Dilute Grote's reagent.*—Dilute one volume of the above reagent with 4 volumes of water before use and take 1 ml of the diluted reagent for a determination. The diluted reagent will keep for a day.

(c) *Thiourea stock soln.*—Dissolve 200 mg of the pure chemical in water and dilute to 200 ml.

(d) *Dilute thiourea soln.*—Dilute 5 ml of reagent (c) to 100 ml with water.

APPARATUS

Siphon.—Insert two bent glass tubes in a two-hole cork or stopper, one terminating just below the stopper and the other long enough to reach the bottom of a centrifuge bottle when the cork with tubes is inserted in the mouth of the bottle. Attach another glass tube to the outside end of the longer bent tube by means of a flexible rubber tube. (The assembly is used to siphon the lower layer from a centrifuge bottle, and the rate of flow is controlled by squeezing the rubber connection.)

Prepare a cap for the inner tube by boring a hole of the same diameter as the tube part way through a small cork.

PREPARATION OF SAMPLE

Juice oranges in an ordinary reamer, strain out seeds and pulp, and mix well. Measure 125 ml into a 250 ml centrifuge bottle, add 70 ml of ethyl ether and shake well for 1–2 min. Centrifuge the bottle and contents for 10 min. at about 1800 r.p.m. and remove. Cap the end of the siphon which is inserted in the bottle and lower it thru the top layer into the lower aqueous layer. Then push off the cork cap with a glass rod. Lower the tube to the bottom of the bottle with cork in mouth and blow in the short tube to start the flow of liquid. Carefully siphon off as much of the lower layer as possible, and control the rate of flow by pressing on the rubber connection. Stop the flow when material from center emulsion layer begins to enter the tube. Add a teaspoonful of filtercel to the siphoned liquid, stir well, and filter on a Büchner funnel with suction, using a Whatman No. 54 or 41 H filter. Warm flask and contents to about 40°C. (on steam bath) and again apply suction to remove ether.

Measure 50 ml of the filtrate into a 150 ml beaker and boil down to about 20 ml. Add 0.5 g MgO and stir until the liquid has a *p*H of about 8.0, as shown by universal indicator paper. Immediately transfer the material quantitatively to a 25 ml volumetric flask, make to the mark, and mix. Filter off the excess MgO immediately, preferably by suction, and pipet 20 ml of the filtrate into a Majonnier flask containing 4 g of salt. Stopper and shake to dissolve salt.

Extract the sample in the tube alternately, first with a 50 ml portion of isoamyl alcohol and then with 50 ml of isoamyl alcohol saturated with water. Shake thoroly each time for 1 min. or longer. Centrifuge in appropriate cups at about 700 or 800 r.p.m. If necessary, add a few glass beads to the tube to bring the interface just to the bottom of the neck after the first extraction. Decant the isoamyl alcohol layer each time into a 500 ml separatory funnel. Make a total of seven extractions in this manner, four with the straight isoamyl alcohol, and three with this solvent saturated with water. (Prepare water-saturated solvent by shaking well about 300 ml with an excess of water, drawing off excess water and filtering the alcohol layer.) Extract the combined alcohol extracts in the funnel four times by shaking well with 120 ml portions of water. Draw off the first aqueous extract into a 400 ml beaker and receive the three succeeding extractions in a separate 600 ml beaker. Add to the contents of the 600 ml beaker 0.1 ml of sirupy (85%) phosphoric acid. Evaporate the contents of the beakers separately by boiling until about 30 ml remains in each. Transfer and combine the contents of the beakers in a glass stoppered 100 ml graduated cylinder. Rinse the beakers in succession two or three times with about 4 ml portions of water. Add 0.4 g of Na citrate, shake to dissolve, and make to a total volume of 80 ml, stopper, and mix.

DETERMINATION

Pipet 10 ml of the final sample soln into a test tube about ⅞ inch in diameter. Prepare standards containing 0, 1, 2, and 4 ml of dilute thiourea soln (reagent d) and make to a total volume of 10 ml with 0.6% Na citrate soln. Add 1 or 2 drops of acetic acid (1 + 5) to each tube and 1 ml of diluted Grote's reagent to each tube with stirring or shaking. Allow the tube to stand 45 min. at 20–25°C. or 15 min. at 45°C. and read the colors (absorption) in a photometer at 610 millimicrons. Construct a graph from the standards, plotting photometer reading or extinction against parts per million of thiourea. (Each ml of standard represents 10 p.p.m. The reading from the sample soln gives directly from the graph the parts per million of thiourea extracted.) Multiply this figure by 1.1 to obtain true thiourea in sample, since only 91% of that originally present is extracted by the procedure.

THIOUREA IN FROZEN PEACHES

REAGENTS

See (a), (b), (c), and (d) of above method for orange juice.

(e) *Sodium bisulfite*.—Prepare a soln containing 2.5 g of $NaHSO_3$ per liter.

PREPARATION OF SAMPLE

Weigh 200–400 g of the frozen sample on a rough balance (0.1–0.2 g sensitivity) into a tared 800 ml beaker. (Cut the contents of a 1 lb. package into quarter or eighth portions and select alternate portions for the determination and keep the remainder as a reserve sample. Several packages can be composited in this manner if desired.)

Immediately weigh into the beaker containing the sample an amount of $NaHSO_3$ soln equal to half the weight of the sample. (See Note 1). Stir the contents of the beaker and pour into a blendor (Waring or other suitable type), drain well, and comminute in the blendor for 20–30 seconds. Return the diluted comminuted sample to beaker.

Weigh 150 g of this blended material to accuracy of 0.2 g and transfer to a 250 ml volumetric flask and dilute to about 200 ml with water. Remove most of the air by adding about 4 drops of hexyl alcohol and inserting a two-hole stopper (No. 0) carrying a small bent glass tube and another straight tube extending about 3 inches into the flask, the end of which is drawn to small bore (near capillary size). Apply gentle suction to the bent tube and shake flask with rotation. The bore of the small tube should be large enough so that reduction of pressure is not too great. If the froth rises in the neck, release vacuum for a moment. Then continue with suction and rotation until most of air is removed, add 20 ml more of sulfite soln and then dilute to the 250 ml mark with water. Mix well and pour about 165 ml into a 250 ml centrifuge bottle. Add 50 ml of ethyl ether to the contents of the bottle, rotate a few times, then stopper and shake, open once to release pressure, than shake vigorously for 1–1.5 min. (If preferred, divide the liquid in vol. flask between two centrifuge bottles and extract each with about 30 ml of ether, etc.)

Place bottle in centrifuge and whirl at about 1800 r.p.m. for about 10 min. Carefully pour off a little of the top ether layer into a beaker, then inclining the bottle, push the sludge cake toward the bottom of the bottle with a glass rod and pour the liquid contents on a cotton filter in a Bunsen funnel. To prepare filter place a small pledget of cotton in the apex of a 85–100 mm funnel, then insert a piece of absorbent cotton of half thickness (split sheet), about 3½ inches in diam.

Pipet 100 ml of the lower aqueous filtrate into a 200 ml volumetric flask. (Squeeze the cotton on the side of the funnel with a rod if necessary to obtain sufficient filtrate.) Add gradually to the contents of the flask (from a separatory funnel), with constant shaking, sufficient acetone to bring the contents to the 200 ml mark. As the surface of the liquid enters the neck of flask, stopper and mix by inverting a few times before making to mark. Mix contents, cool to room temperature (in bath if desired), make to mark again, and mix well. (Note: Acetone causes some rise in temperature.) Let precipitate separate and pour contents of flask into a 250 ml centrifuge bottle. Add a spoonful of filtercel, stopper, and shake well, then centrifuge about 8 min. at about 1800 r.p.m. Decant off the supernatant liquid and filter if unclear. Measure 125 ml of the clear liquid into a 250 ml beaker, add several glass beads and boil off the acetone on the steam bath. Then boil down to about 35 ml on the hot plate, remove and cool to room temperature. Add dropwise a 15% soln of NaOH until basic and about 2 drops in excess. Add acetic acid (1+5) with stirring until just acid and add 2 drops in excess.

Transfer the liquid quantitatively to a 50 ml volumetric flask and make to mark with water and mix. Pour the contents of the flask into a small beaker or flask (100–125 ml), add a spoonful of filtercel, and mix well by stirring or stoppering and shaking. Filter the liquid on a 12.5 cm folded filter (E. & D. No. 195 is suitable). Pour through filter again if not clear. The filtrate or final sample soln is designated F. S.

DETERMINATION

Pipet 10 ml of soln F. S. into a 6-inch test tube. For expected amounts of thiourea below 20 p.p.m., prepare standards containing 0, 1, and 2 ml portions of soln **(d)** (5 mg thiourea per 100 ml). For amounts from 20 to 50 p.p.m., prepare standards containing 0, 2, and 4 ml of standard soln **(d)**. Add 0.6% Na citrate to the tubes to make the standards to 10 ml volume, then add 1 drop acetic acid (1+5) to each tube (samples and standards). Place a stirring rod in each tube and stir up and down to mix, leaving rod in tube. Place the tubes in a bath or a room maintained at a temperature of 20–25°C.

Add with stirring 1 ml of recently diluted Grote's reagent **(b)** to each tube. Allow the tubes to stand at the above temperature for 60 min. and read the colors (blue) of each tube in a photometer, using a 1-inch cell and a filter centering at about 610 millimicrons. Designate the reading of the sample as X. Construct a lineal curve from the standard readings, plotting parts per million of thiourea (1 ml stand. = 10 p.p.m.) against photometer readings. Extrapolate the curve made with 2 and 4 ml portions of standard soln for amounts up to 50 p.p.m.

A correction of the reading (X) obtained above is necessary since the soln naturally contains some color before the reagent is added. To do this make readings of the soln F. S. with no added reagent in the same cell and also of distilled water. Obtain the difference of (F. S.) reading − distilled water reading = **(d)** and subtract **(d)** from the reading X above. X − d = R = corrected reading. From the reading (R) obtain the thiourea in p.p.m. in the sample soln (F. S.) by use of the curve. Multiply the thiourea thus found by the factor 1.065 to correct for the volume increase due to ether and obtain the true thiourea content of the original sample. (See Note 2). Repeat the determination (color development) on a smaller aliquot (1–5 ml) for quantities greater than 50 p.p.m.

NOTE 1

Two hundred g is sufficient sample to be representative and should be used where a portion is to be reserved. The unused portion of the sample should be maintained in a frozen condition. It is necessary to add the sodium bisulfite soln immediately to the frozen sample before blending to prevent losses of thiourea due to attack by the enzymatic systems present. Blending in the Waring blendor whips air throughout the material and if the enzymes are not inactivated, large losses of thiourea are likely to occur. The enzymes can also be inactivated by plunging the frozen sample into boiling water and boiling 3 or 4 min. The action of the enzymes is slow in the frozen condition where the material is in unbroken cakes or chunks.

NOTE 2

The correction factor 1.065 is to compensate for the solubility of ether in the aqueous soln. Measurement of 100 ml of soln is made after extraction with ethyl ether. The latter has considerable solubility in aqueous solutions. In previous methods the presence of NaCl in the soln reduced the solubility of the ethyl acetate to a large extent and correction was not necessary for this reason.

(3) The following tests were adopted as tentative.

QUALITATIVE TESTS FOR PRESENCE OF THIOUREA IN ORANGE JUICE

(*A*) *Using the Pentacyanoammonioferrate*

Reagent.—Preparation according to Fearon.

Dissolve 10 g of sodium nitrosoferricyanide (nitroprusside) in 40 ml of conc. NH_4OH soln (sp. gr. 0.88) and keep at about 0°C. until all the nitrosoferricyanide has decomposed. This is shown when a few drops of the mixture no longer give a red color when added to a solution of creatinine in *N* NaOH. Decomposition is complete by the end of 24 hours. Remove the precipitate by filtration and precipitate the residual pentacyanoammonioferrate in the soln by addition of absolute ethanol until no further precipitate appears. Collect the precipitate, wash with absolute ethanol until free of ammonia, and dry in vacuo over sulfuric acid. Keep the solid reagent in a desiccator over $CaCl_2$ in the dark.

Prepare a 1% soln of the solid in distilled water, expose it to light and air for a day, and then store in a brown glass bottle in the dark. The reagent is now ready for use, gains in potency for several weeks, and can be kept for about six months.

PREPARATION OF SAMPLE

Extract a volume of juice with about two thirds its volume of ethyl ether, centrifuge, and separate lower layer. Stir in some filtercel and filter with suction. Keep vacuum on for a short time and agitate to remove most of ether.

To about 5 ml of the extracted sample add 5 drops of the reagent above. Note color. If a blue color does not develop, add about 0.1 *N* iodine soln a drop at a time, shaking after each drop. Usually, about 5 drops are necessary to develop maximum color (blue green). Excess iodine tends to reduce the color.

(*B*) *Qualitative Test with Grote's Reagent*

Reagent.—Use that given above in quantitative method for orange juice (a), p. 100.

TEST

Use extracted sample prepared in (*A*) above.

To about 5–10 ml of prepared sample add .02 *N* iodine soln dropwise until a drop remains and does not disappear for some time. Add a ml or so of diluted Grote's reagent. A blue green or blue color develops rather gradually in the presence of thiourea.

(4) The following method was adopted as official, first action.

DETERMINATION OF MONOCHLORACETIC ACID

(Applicable to carbonated beverages and fruit juices containing 5–150 mg CH_2Cl COOH in 150 ml).

APPARATUS

Continuous extractor (**39.112, Fig. 15**).—Make the outer part 45 cm long from 43 mm tubing with side tube issuing 25 cm above the bottom, and fitted with standard taper, drip tip, joint 24/40. Make the inner tube 40 cm long from 12 mm tubing. Use a 250 ml conical flask with suitable joint to hold the overflowing ether extract. Any equally efficient means of extraction may be used.

REAGENTS

Silver nitrate soln.—(1 ml = ±5 mg CH_2Cl COOH.) Dissolve 9 grams of $AgNO_3$ in water and dilute to 1 liter.

Ammonium sulfocyanate soln.—(1 ml = ±5 mg CH_2Cl COOH.) Dissolve 4.03 g of NH_4CNS in water and dilute to 1 liter. Standardize against pure NaCl soln, 3.093 g per liter, which contains 1.8762 g of Cl (equivalent to 5 g of monochloroacetic acid, which contains 1.8764 g of Cl).

Ferric indicator.—A saturated soln of ferric ammonium alum.

DETERMINATION

In the outer part of the continuous extractor, place such a quantity (not over 150 ml) of carbonated beverage, apple juice, grapefruit or orange juice as contains 5–100 mg of CH_2Cl COOH. Dilute if necessary to 150 ml, add 3–5 ml of H_2SO_4, mix, and extract with ether 2–3 hours. Tilt the extractor in such a way as to drain as much as possible of ether into the flask; disconnect the flask, add 25 ml 1 N NaOH in excess of that required to make the water layer alkaline to litmus paper after shaking, shake, and evaporate the ether on the steam bath until only about 25 ml of liquid remains, hastening the process by passing a current of air into the mouth of the flask. Digest on the steam bath for 2 hours or boil under a reflux condenser for ½ hour. Add 50 ml of water, 15 ml of HNO_3, and a known volume of the $AgNO_3$ soln in excess. Shake ½–1 min, add the ferric indicator, and titrate the excess Ag with the NH_4CNS soln. In the titration, add NH_4CNS soln carefully until the pink color formed fades slowly on mixing. At this point shake the soln for ca 30 seconds and filter thru a folded filter into a second flask. When the first flask is empty wash down its walls with ca 50 ml of H_2O and add this to the filter after the soln has all gone thru. When the wash water has passed thru, complete the titration. In the same way, titrate a quantity of $AgNO_3$ soln equal to that added to the sample. The difference between the two titrations is a measure of the CH_2Cl COOH.

(5) The following methods were adopted as official, first action.

QUATERNARY AMMONIUM COMPOUNDS

I. Ferricyanide Method.

(For commercial preservatives)

REAGENTS

Buffer.—Dissolve 130 g of sodium acetate in water, add 42 ml of acetic acid and make up to 500 ml.

Ferricyanide solution.—Dissolve 6.6 g of $K_3Fe(CN)_6$ in water and dilute to 1 liter.

Zinc sulfate solution.—Disolve 20 g of $ZnSO_4\cdot 7H_2O$ in 180 ml of water.

Thiosulfate solution.—Dissolve 5 g of $Na_2S_2O_3\cdot 5H_2O$ in water, dilute to 1 liter and standardize (1 ml of 0.02 N =0.02142 g alkyldimethylbenzylammonium chloride, molecular weight 357.

Approximation of Quaternary Ammonium Salt Content

Pipet 1 ml of buffer, 2 ml of ferricyanide solution, and 20 ml of water into each of 4 small conical flasks. Pipet into them, respectively, 0.5 ml, 1.0 ml, 2.0 ml, and 4.0 ml of sample, mix and filter. Now add 2 ml of sample to each filtrate, mix, and observe.

DETERMINATION

Into a 100 ml Kohlrausch flask[1] pipet such an aliquot of sample as contains about 0.5 g of quaternary ammonium salt as indicated in Table 1, dilute if necessary to make the volume 50 ml, add 5 ml of buffer and mix. Then add from a pipet 30 ml of ferricyanide solution, rotating the flask during the addition. Dilute to the mark

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[1] In case of a solution containing approximately 0.5 g of the compound per 100 ml, pipet 100 ml into a 200 ml volumetric flask, use 10 ml of buffer, and make the back titration upon 100 or 150 ml of filtrate.

TABLE 1.—*Approximation of content of alkyldimethylbenzylammonium chloride (mol. wt. 357)*

	SAMPLE ADDED			
	A 0.5 ML	B 1.0 ML	C 2.0 ML	D 4.0 ML
Per cent				
8.4 or more	No ppt.	No ppt.	No ppt.	No ppt.
5	Ppt.	No ppt.	No ppt.	No ppt.
2.5	Ppt.	Ppt.	No ppt.	No ppt.
1.25	Ppt.	Ppt.	Ppt.	No ppt.
1 or less	Ppt.	Ppt.	Ppt.	Ppt.

with water and mix. After ½ hour, filter, discarding the first 10–15 ml of filtrate. Pipet 50 ml of filtrate into a 500 ml conical flask, add 100 ml of water and 1–2 g of solid KI. Rotate until the salt is dissolved; add 10 ml of HCl (1+1), mix, and let stand for 2 min. Add 10 ml of zinc sulfate soln, mix, and titrate with thiosulfate soln, adding starch indicator when the iodine color fades to a tinge of yellow. Run a blank determination including all of the above operations but substituting water for the sample. Calculate the quaternary ammonium salt content from the difference in the two titrations.

II. Method for Bottled Beverages Containing Fruit Juices

APPARATUS

Centrifuge having a head about 12 inches in diameter equipped for accommodating 100 ml portions of sample.

Steam distillation apparatus (see 25.77): Use 500 or 1000 ml distillation flask fitted with spray tube reaching to within 1 or 2 cm of the bottom of flask (all connections standard taper joints), and with plug for steam inlet (to be used during the early part of the distillation).

Photometer: fitted with color filter 610 mμ and suitable cell.

REAGENTS

Sodium carbonate solution.—Dissolve 5 g Na_2CO_3 in 500 ml of H_2O.

Bromophenol blue.—Use the solid reagent.

Bromophenol blue solution.—Dissolve 40 mg of the dry compound in warm H_2O, cool, and dilute to 100 ml.

Sodium sulfate.[1]—Anhydrous granular (not powdered) reagent grade.

Solvents.—Ethylene chloride; petroleum ether.

DETERMINATION

(a) *Extraction.*—Mix thoroly, and measure out 50 ml of sample in a graduated cylinder. Filter on a 7 cm Büchner funnel and make the filtrate to 100 ml with water (Soln A). Place the filter paper in a 400 ml beaker and extract with small portions of alcohol until no more color is extracted and the paper remains white. Transfer the alcoholic extract to a 500 ml distilling flask, add 10 mg of bromophenol blue, 2 ml of HCl (1+1) and 100 ml of H_2O. Steam distil as before, collecting a volume of distillate which is at least 100 ml greater than the volume of alcohol in the extract. Cool the residue, transfer to a separatory funnel and wash with 40 ml, 30 ml, and 30 ml portions of petroleum ether; pipet 50 ml of ethylene chloride into the separatory funnel and shake for 3–4 min. Let stand until clear and draw off the lower layer into a second separatory funnel containing 10 ml of sodium carbonate soln, and shake for 3–4 min. When the two layers separate, observe the lower one. If blue, a quaternary base is present. Judge from the depth of color whether or not it is suitable for reading in the photometer. If so, draw off the lower layer into a glass-stoppered flask containing 1–2 g of anhydrous granular Na_2SO_4 and, after 30 min, read as before in a suitable cell using a filter centering at 610 mμ.

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[1] Mallinckrodt Sodium Sulfate Anhydrous granulated A.C.D. was found to be satisfactory.

If the color is too deep, acidify the contents of the second separatory funnel with 1 or 2 ml of HCl (1+1), shake until the contents become yellow, and return to the first separator. Pipet a second 50 ml portion of ethylene chloride into the first separator, shake 3–4 min and let stand until the lower layer is clear. (If the sample is known to contain over 1 mg of quarternary ammonium compound in 100 ml, add the entire 100 ml of ethylene chloride in the first instance.) Now follow the directions for (c) "*Measurement of Color.*"

Meanwhile, pipet a suitable aliquot (first try 5 ml) of soln A into a separatory funnel, add 3 ml of bromophenol blue soln and 1 ml of HCl (1+1); and proceed as above beginning "pipet 50 ml of ethylene chloride * * *."

(b) *Preparation of the Standard Curve.*—Standardize a 1% soln of the quaternary ammonium compound to be determined, using the ferricyanide method. By carrying out the procedure given below, ascertain the maximum and minimum concentrations of the chemical which produce, in 50 ml of ethylene chloride, colors of a density suited to the color-measuring instrument to be employed. Now prepare a set of three or more standards containing, in 50 ml quantities of the quaternary ammonium compound covering the range between these two points. (If the neutral wedge photometer is to be used, 0.0 mg, 0.1 mg, 0.2 mg, and 0.25 mg per 50 ml are suitable standards.)

Pipet 50 ml of each standard into a series of separatory funnels, add to each 3 ml of bromophenol blue solution and 1 ml of HCl. Pipet 50 ml of ethylene chloride into each and shake for 2–3 minutes. When clear, draw off each lower layer into a series of funnels containing 10 ml of sodium carbonate solution, and shake for 2–3 min. Let stand until clear, draw off into a glass-stoppered flask containing 1–2 g of granular anhydrous Na_2SO_4 and, after 30 minutes, read in the color-measuring instrument, using the same (or a similar) cell and a light filter centering at 610 mμ (a 1" cell was used in the neutral wedge photometer). If the instrument reads in terms of per cent transmission, the proper conversion to color density must be made. If, however, it reads directly in color density, or in scale readings which are proportional to the density of color (as in the case with the neutral wedge photometer), the readings may be plotted directly against the concentrations employed.

(c) *Measurement of color.*—Treat each ethylene chloride extract *separately*[1] as follows: Introduce 30–50 ml of the extract into a separatory funnel containing 10 ml of sodium carbonate solution. Shake for 3–4 min. When clear, draw off the lower layer into a glass-stoppered flask containing 1–2 g of anhydrous granular Na_2SO_4. After 30 min read in a suitable cell using a filter centering at 610 mμ.

If the color is too deep for accurate reading, estimate a suitable volume of the extract to be used as an aliquot; pipet this volume into a 50 ml volumetric flask and fill to the mark with ethylene chloride. Shake the mixture with sodium carbonate solution, separate, dry, and read as above. From the quantities of quaternary ammonium compound found in the extracts from the liquid and the solid materials, calculate to mg per 100 ml of fruit juice.

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[1] When the two ethylene chloride extracts were united for this step, lower results were obtained.

III. Method for Beer

Place 100 ml of decarbonated beer in the steam distillation flask; add 10 mg of bromophenol blue and 2 ml of HCl (1+1). Steam distil, collecting about 200 ml of distillate. Cool the residue, transfer to a separatory funnel, wash with 100 ml and then with 50 ml of petroleum ether. Then proceed as directed in the method for Bottled Beverages Containing Fruit Juices, beginning "pipet 50 ml of ethylene chloride, ..."

IV. Method For Table Sirup

Weigh 20 g of sample, transfer to a 100 ml volumetric flask, mix thoroly, and make to volume with water. Pipet an aliquot of the soln into a separatory funnel; add 5 ml of bromophenol blue soln, 1 ml of HCl (1+1) and proceed as directed in the Method for Bottled Beverages Containing Fruit Juices, beginning "pipet 50 ml of ethylene chloride, ..."

33. SPICES AND CONDIMENTS

(1) The tentative method for caramel in vinegar, section **33.78** (p. 553), was deleted.

(2) The following method for starch in mayonnaise and salad dressing was adopted as official, first action.

STARCH IN MAYONNAISE AND SALAD DRESSING

REAGENTS—*See* **33.41**

DETERMINATION

Determine the total acidity of the prepared sample by **33.51**. Place 4–5 g of the prepared sample in a 500 ml Erlenmeyer flask, add the calculated quantity of 0.1 *N* NaOH to neutralize the acid in the weight of sample taken, and add 100 ml of the $CaCl_2$ soln from a pipet. Stopper the flask and swirl gently until all large lumps of dressing are broken up. Continue as in the method for starch in mustard products, **33.42**, beginning "Add glass beads. Connect to reflux condenser . . . ." Calculate percentage of starch from the formula:

$$\%\ \text{Starch} = \frac{\text{g. dextrose} \times 0.9\ (100 - A) \times 8}{\text{weight of sample}}$$

where A = ml 0.1*N* NaOH used to neutralize acidity of the sample.

(3) The tentative method for starch in mustards, *This Journal*, **30**, 75 (1947), was adopted as official, first action, after the following changes:

(a) Delete the sentence "Filter . . . funnel," lines 10 and 11, and substituting "Filter thru an 11 cm circle of absorbent cotton ca ½ cm thick placed in a 60° funnel."

(b) Adding "several glass beads and" between "Add" and "water," line 16.

(c) Adding "as possible" after the word "dispersed," line 17.

(d) Correcting "or" to "on" in parenthesis, last line.

(e) Changing "0.8" to "8.0" in the numerator of the formula for calculating the percentage of starch.

(4) The official method for copper-reducing substances in prepared mustard, section 33.40 (p. 545) was deleted, final action.

34. SUGARS AND SUGAR PRODUCTS

(1) The following method was adopted as tentative:

UNFERMENTED REDUCING SUBSTANCES IN MOLASSES

*Method of Java Sugar Experiment Station**

REAGENTS

(a) *Baker's yeast, free from starch.*—Fleischmann's, sold in packages of 1 lb. each by Standard Brands, Inc., was found suitable. It keeps fresh for a few days if kept in the refrigerator.

(b) *Neutral lead acetate soln.*—Dissolve 20 g $Pb(C_2H_3O_2)_2 \cdot 3H_2O$ in H_2O to 100 ml.

(c) *Anhydrous potassium oxalate.*

(d) *Soxhlet soln.* See section **34.33** (a) and (b).

(e) *Potassium iodide soln.*—Dissolve 20 g KI in H_2O to 100 ml.

(f) *Dilute sulfuric acid.*—5 volumes of water plus 1 volume of conc. H_2SO_4.

(g) *Standard thiosulfate soln.*—0.1 *N*.—See sections **43.28** and **43.29**.

FERMENTATION

Transfer 12 g of blackstrap molasses (or 8 g High-test molasses) to a 500-ml volumetric flask, using in all 75 ml of H_2O. Add 25 g of coarsely chopped, fresh baker's yeast and mix thoroughly with the molasses soln. Close the flask with a stopper provided with a delivery tube the other end of which dips about 1 cm below the surface of H_2O in a beaker; or use any other type of fermentation trap. Place the flask in a water bath kept at 30°C. and allow to ferment for at least 4 hours, shaking the flask from time to time. When fermentation is complete dilute with H_2O, clarify with 15 ml of neutral $Pb(C_2H_3O_2)_2$ soln, make to the mark at 20°C., add a teaspoonful of Filter-Cel, shake well and filter, discarding the first runnings. Delead the entire filtrate with ca 0.5 g of anhydrous $K_2C_2O_4$ and filter again with the aid of Filter-Cel. Test the filtrate for Pb. If necessary add more oxalate and refilter.

DETERMINATION

Transfer 25 ml of the final filtrate to a 250-ml Erlenmeyer flask, mix with 20 ml of combined Soxhlet soln, and wash the wall of the flask down with 5 ml of H_2O, making 50 ml in all. Add a few pieces of ignited pumice stone and place the flask on a wire gauze covered with an asbestos plate which has a hole in the center, slightly smaller than the bottom of the flask. As the source of heat use either a Bunsen burner or preferably an electric heater with temperature control. Heat to boiling in 3 min and boil gently for exactly 2 min longer. Close the flask immediately with a stopper provided with a Bunsen valve and cool quickly under the water tap to prevent reoxidation. Add 15 ml of KI soln and then 10 ml of dilute H_2SO_4. Titrate the liberated I at once with 0.1 *N* $Na_2S_2O_3$ soln, adding starch indicator toward the end of the titration.

Run a blank with 75 ml of H_2O instead of molasses soln, adding yeast, etc., as described. Deduct the titer of the sample from the titer of the blank, and find the mg invert sugar corresponding to the difference, from the table. The result, divided by 6 (in the case of High-Test molasses, by 4) gives directly the percent of unfermentable reducing substances in the molasses, in terms of invert sugar.

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* "Methoden van Onderzoek bij de Java-Suikerindustrie," 6th ed. (1931), p. 365.

Milligrams of Invert Sugar Corresponding to Milliliters of 0.1 *N Thiosulfate*

0.1 *N* THIOSULFATE	INVERT SUGAR	0.1 *N* THIOSULFATE	INVERT SUGAR
ml	*mg*	*ml*	*mg*
1	3.2	14	47.3
2	6.4	15	50.8
3	9.7	16	54.3
4	13.0	17	58.0
5	16.4	18	61.8
6	19.8	19	65.5
7	23.2	20	69.4
8	26.5	21	73.3
9	29.9	22	77.2
10	33.4	23	81.2
11	36.8	24	85.2
12	40.3	25	89.2
13	43.8	—	—

(2) The following changes were adopted in sections **34.18** (p. 560), as official, final (special action).

Change **34.18(a)** to **34.18(b)**; delete the second paragraph beginning "Verification" and the third paragraph beginning "According"; delete in sixth paragraph on page 561, line 4, "at 20° before reading is made." and substitute "at the same temperature (preferably 20°) when the observation is made," and delete "recently" in next to last line; delete in eighth paragraph, page 561, "basic acetate of lead . . . " and substitute "dry basic lead acetate (Horne's Dry Lead, or **34.19(c)**)"; delete ninth paragraph beginning "Whenever reducing"; delete tenth paragraph beginning "After" and substitute "Bring the soln exactly to mark at proper temp., and after wiping out neck of flask with filter paper, add minimum amount of dry basic lead acetate (**34.19(c)**), shake to dissolve, pour all the clarified sugar soln on rapidly acting, air-dry filter. Reject at least first 25 ml of filtrate and use remainder, which must be perfectly clear, for polarization."

Change **34.18(b)** to **34.18(c)** delete "Ventzke" lines 2 and 3 and substitute "Herzfeld-Schönrock"; delete "Bureau of Standards" lines 4, 5, and 6 and substitute "International Sugar."

(3) The following new subsection was adopted as official, final (special action), as section **34.18(a)**.

"The saccharimeter scale shall be graduated in conformity with the International Sugar Scale adopted by the International Commission for Uniform Methods of Sugar Analysis. Rotations on this scale shall be designated as degrees sugar (°S).

The basis of calibration of the 100° point on the International Sugar Scale is defined as the polarization of the normal solution (26.000 grams of pure sucrose dissolved in 100 ml solution and polarized at 20° in a 200 mm tube, using white light and the dichromate filter as defined by the Commission). This solution, polarized in room or cabinet, temperature of which is also 20°, must give saccharimeter reading

of exactly 100°S. Temperature of the sugar soln during polarization must be kept constant at 20°.

The following rotations shall hold for the normal quartz plate of the International Sugar Scale:

Normal Quartz Plate = 100°S = 40.690° ±0.002°.
(λ = 5461A) at 20°.
1° (λ = 5461 A) = 2.4576°S.
Normal Quartz Plate = 100° S = 34.620° ±0.002°.
(λ = 5892.5A) at 20°.
1° (λ = 5892.5A) = 2.8885°S.

According to determination of Bates and Jackson (7)* at the National Bureau of Standards, the Herzfeld-Schönrock scale reading for 26 grams of pure dry sucrose under the above conditions is 99.89_5. The scale reading has been redetermined by Balch and Hill (8)* and by Zerban and Hardin (8)* who found values of 99.907° and 99.912°, respectively. Average value of these independent investigations is 99.90_5°. The value 99.90° was adopted by the International Commission in 1932.

In the case of existing instruments graduated on the Herzfeld-Schönrock scale, it shall be permitted either to change the saccharimeter scale or to use a weight of 26.026 g in 100 ml."

(4) The following change was adopted as section **34.19** (p. 562) as official, final (special action).

Delete in subsection **(a)** last sentence in parenthesis and substitute "(This reagent is not recommended for clarifying products of low purity, due to error caused by volume of precipitate)"; delete in subsection **(c)** last sentence in parenthesis and substitute "(Unless in excess, dry basic lead acetate does not cause a volume error)"; delete subsection **(e)** and substitute the following:

"**(e)** To remove excess lead used in clarification add to clarified filtrate anhydrous K or Na oxalate in small quantities, until test for Pb in filtrate is negative; refilter."

(5) The "**(e)**" in section **34.23(a)** (p. 565) line 3, was deleted, official, final (special action).

(6) The reference "Int. Sugar Jour. **35**, 19 (1933); **39**, 32s (1937)" was added to selected reference (6) p. 594.

35. PROCESSED VEGETABLE PRODUCTS

No additions, deletions, or other changes.

36. VITAMINS

(1) The following method was adopted as alternate tentative.

CAROTENE

EXTRACTION

(a) *Hays and dried plants.*—**Grind the sample to pass a 40-mesh sieve. Weigh accurately a 2-g sample (4 if carotene content is low) and place in the flask of an extractor (Goldfisch, Bailey-Walker, or ASTM is suitable if no thimble is used). Add 30 ml of a 3:7 mixture of acetone and commercial hexane (therefore 30% acetone) to the flask and reflux the contents at a rate of 1–3 drops per second for one hour.**

*** Reference numbers refer to selected references, *Methods of Analysis*, 6th Ed., p. 594.**

After cooling to room temperature, decant or filter the extract into a 100 ml volumetric flask, wash the residue with hexane, and dilute the soln to volume. The soln, which now contains 9% acetone, is chromatographed.

CHROMATOGRAPHY

Prepare a chromatographic column with a 1:1 mixture of activated magnesia (Micron brand No. 2642, Westvaco Chlorine Products Company, Newark, California) and diatomaceous earth (Hyflo Supercel, Johns-Manville, Chicago, Illinois). A suitable chromatographic tube can be made from a Pyrex test tube 22 mm O.D. and 175 mm long by sealing a smaller tube (ca 10 mm O.D.) in the bottom. To prepare the chromatogram place a small plug of glass wool or cotton inside the tube, add the loose adsorbent to a depth of 15 cm, attach the tube to a suction flask, and apply the full vacuum of a water pump. Use a flat instrument such as an inverted cork mounted on a rod to gently press the adsorbent and flatten the surface. The packed column should be about 10 cm deep. Place 1 cm. layer of anhydrous sodium sulfate above the adsorbent.

With the vacuum continuously applied to the flask pour the extract into the chromatographic column and use 50 ml of a 1:9 mixture of acetone and hexane to wash the carotene into the adsorbent and develop the chromatogram. Keep the top of the column covered with a layer of solvent during the entire operation. This is conveniently done by clamping an inverted volumetric flask full of solvent above the column with the neck 1–2 cm above the surface of the adsorbent.

The carotenes pass rapidly through the column and the entire eluate is collected. Bands of xanthophylls, carotene oxidation products, and chlorophylls should be present in the column when the operation is complete. Transfer the eluate, which has been reduced in volume by loss of vapor through the water pump, to a 100 ml volumetric flask and dilute to volume with a 1:9 mixture of acetone and hexane. The carotene content of this soln is determined photometrically.

PHOTOMETRY

Determine the density of the solution as soon as possible with a spectrophotometer at 436 mμ or with some other instrument which is provided with a suitable filter system, such as the Klett photometer with a No. 44 filter, or the Evelyn photoelectric colorimeter with a 440 filter. These instruments are first calibrated with solns of beta-carotene of high purity as shown by the characteristic absorption curve (See Beadle and Zscheile, *J. Biol. Chem.*, **144**, 21 (1942), for characteristic curve). A calibration chart is prepared and the density of the soln to be determined is then converted into carotene concentration by referring to the chart.

When determinations are made with the Beckman spectrophotometer at 436 mμ, the specific absorption coefficient (*alph* = 196) is used in the formula

$$C = \frac{\log\frac{I_0}{I} \times V \times 1000}{\cdot 196 \times L \times W}$$

where C is concentration of carotene in parts per million contained in the original sample, V is the final volume of the eluate at time of reading, L is length of cell in cm, and W is the weight of sample. Report results as parts per million of beto-carotene.

(2) The tentative fluorometric method for vitamin B (thiamine), sections 36.24–36.26 (p. 608), was adopted as official, first action.

37. WATERS, BRINE, AND SALT

No additions, deletions, or other changes.

38. RADIOACTIVITY

No additions, deletions, or other changes.

39. DRUGS

(1) The following method for ephedrine was adopted as official, first action.

EPHEDRINE

(a) *Oily inhalants containing oxazolidines (products from the reaction of ephedrine with carbonyl compounds).*—Accurately weigh or otherwise measure out a sample containing ca 100 mg of ephedrine. (In the case of most inhalants, the sample size will be about 10 ml. Although a sample containing as little as 20 mg of the drug may be used, if necessary, the larger sample should be employed if practicable.) Rinse it quantitatively into a 125 ml Erlenmeyer flask with portions of reagent grade benzene totalling about 5 ml, add 10 ml. of 5% sulfuric acid, and frequently agitate and swirl the mixture while it is boiled gently for 10 min. The boiling is best done on a hot plate and should be conducted carefully to avoid superheating, bumping, and loss of sample. Cool the flask, transfer the contents to a 125 ml separatory funnel, and rinse the Erlenmeyer with portions of benzene totalling about 1½ times the volume of sample in order to remove all oily matter from the flask. (It is desirable that the Erlenmeyer be provided with a lip in order to facilitate the quantitative transfer of the contents to the funnel.) Shake the funnel containing the acid and benzene rinsings, drain off the acid layer into a second separatory funnel, and swirl the first funnel vigorously to force down additional acid and insure more complete separation of the phases. Drain into the second funnel any acid layer which further separates, and extract the benzene-oil phase with three 5-ml portions of water which have been previously used to rinse the flask. Swirl the first funnel each time after the main portion of water has been drained into the second. (In the transfer of ephedrine from organic solvent to aqueous phase, and *vice versa*, shakeouts should be conducted for at least 1 min and in as vigorous manner as possible without causing troublesome emulsions.) Wash the acid soln of ephedrine sulfate with 3 ml of chloroform, and discard the chloroform washings. Make the soln alkaline to litmus with 20% sodium hydroxide (about 2.5 ml is required), then add 0.5 ml in excess and extract the ephedrine by shaking *vigorously* with six 15-ml portions of U.S.P. chloroform. If more than 50 mg of ephedrine is present, filter the extracts through a pledget of cotton into a tared 100 ml beaker previously dried at 110° and cooled in a desiccator. After the fourth extraction, rinse the filter funnel and its tip with chloroform (allowing the rinsings to drain into the beaker), float 5 drops (0.2 ml) of conc. hydrochloric acid on the surface of the combined extracts, and evaporate on the steam bath in a current of air until the beaker can easily accommodate the remaining extracts. To test for complete extraction, shake the alkaline phase with a seventh and an eighth 15-ml portion of chloroform, filter these through cotton into a small beaker, float 2 drops of conc. hydrochloric acid on the surface, and evaporate to dryness on the steam bath in a current of air. If a *crystalline* residue results, combine it with the main extracts by using a little reagent grade methanol for the transfer, and repeat the test if considered necessary. Continue the evaporation of the main extracts until the volume of liquid has been reduced to about 1 or 2 ml. Then cautiously heat on the bath, but without the air current, until the odor of hydrogen chloride has disappeared, and the residue of ephedrine hydrochloride is apparently dry. Heat the beaker in an oven at 110° C. for ½ hour, cool in a desiccator, and weigh: Weight of residue X 0.8192 =

weight of ephedrine base. If less than 50 mg of ephedrine is present in the sample, carry out the extraction of ephedrine to completion as described above, and evaporate the filtered extracts in an untared beaker until the chloroform, but not the excess hydrochloric acid, has been removed. Direct a fine stream of redistilled reagent grade methanol around the inside of the beaker to dissolve the ephedrine salt, and immediately repeat the process with a stream of chloroform. Transfer the methanol-chloroform soln to a tared 20 ml beaker, previously dried at 100° and cooled in a desiccator, and repeat the methanol and chloroform rinsings until the ephedrine hydrochloride has been quantitatively transferred. Evaporate the soln on a steam bath, in a current of air, until the salt begins to crystallize. Continue the removal of solvent by cautious heating alone, to avoid loss from crepitation, until the residue is apparently dry and there is no odor of hydrogen chloride. Dry and weigh as previously described.

(b) *Oily inhalants or petroleum jelly preparations containing free ephedrine only.* If the product is an oil, quantitatively transfer a suitable sample (see **a**) to a 125 ml separatory funnel with the aid of portions of benzene totalling about 1½ times the volume of sample, and extract the mixture with 5 ml of 10% sulfuric acid, then with four 5-ml portions of water, swirling the funnel each time as in (**a**), and continue the assay as described in that paragraph beginning at the point "Wash the acid solution of ephedrine sulfate. . . . "

If the product is a petroleum jelly preparation, dissolve the sample in sufficient benzene to obtain a soln of suitable fluidity (30 ml should be sufficient for a sample of 10 g) and proceed with the assay as described for oily inhalants.

(c) *Water-soluble jellies.*—If the product is a thin jelly of viscosity similar to that of the N. F. VIII preparation, accurately weigh a sample of about 10 g. If it is a thick jelly, reduce the size of the sample (which should contain 20–100 mg of the alkaloid) to about 5 g to diminish the possibility of emulsion formation during the ether extractions. Transfer quantitatively to a separatory funnel with the aid of water, and dilute with the latter until the volume of jelly plus water is about 20 ml. Render the mixture slightly alkaline to litmus with 20% sodium hydroxide, add an additional one-half ml, and quantitatively extract the ephedrine with U.S.P. ether according to the usual three-funnel technic. In each shakeout use a volume of ether equal to that of the aqueous phase, and swirl the funnel vigorously after draining off the bulk of the aqueous layer in order to obtain an efficient separation of the two phases. If equilibrium is attained in each shakeout, 5 or 6 will be sufficient. Extract the ether with 5 ml of 10% sulfuric acid and four 5-ml portions of water in the same manner as described for the extraction of the benzene-oil mixture in (**b**), and continue the assay from this point according to the directions of that section.

(d) *Syrups.*—Use a 10 ml sample, measured by means of a volumetric flask, and proceed as directed under (**c**).

(e) *Solutions of ephedrine salts.*—Use an accurately measured volume of sample containing about 100 mg of ephedrine and carry out the assay as directed under (**c**), except that if the sample amounts to less than 20 ml it need not be diluted to this volume before extracting.

(f) *Tablets and capsules.*—Accurately weigh a sample of the capsule contents or finely powdered tablets preferably equivalent to about 100 mg of the drug. Transfer to a separatory funnel containing 20 ml of water and basify with ca 0.5 g of anhydrous sodium carbonate. Proceed with the assay as described under (**c**), beginning with the ether extractions, but basify the acid extracts with anhydrous sodium carbonate instead of 20% sodium hydroxide. Use ca 0.5 g in excess of that required to make the soln alkaline to litmus.

(2) The following method was adopted as official, first action.

SPIRIT OF CAMPHOR

REAGENT

2, *4-dinitrophenylhydrazine*.—Dissolve 2 g of 2, 4-dinitrophenylhydrazine in 20 ml of 1+1 cold H_2SO_4 by shaking in a glass-stoppered flask; add 35 ml of water, mix, cool, and filter.

DETERMINATION

Dilute an accurately measured quantity of spirit of camphor with aldehyde-free alcohol so that the soln contains ca 0.2 g of camphor per 10 ml. Pipet 10 ml of the dilution into a 125 ml pressure bottle containing 50 ml freshly prepared dinitrophenylhydrazine reagent. Close pressure bottle, immerse it in a beaker of water, and heat on steam bath 4 hours. (Temperature of pressure bottle should be maintained ca 75°C.)

Cool the bottle and contents to room temperature, then transfer contents to a beaker using 100 ml 1+11 H_2SO_4. Allow to stand overnight at room temp. Collect the precipitate on a tared Gooch crucible, wash with 10 ml 1+11 H_2SO_4, then with 75 ml cold water to remove the acid. Dry at 100°C. The weight of precipitate $\times .458 =$ weight of campher.

(3) The following method was adopted as official, first action.

QUINACRINE HYDROCHLORIDE (ATABRINE)

REAGENTS

Sodium acetate-acetic acid mixture.—Dissolve 2.5 g sodium acetate and 1 ml glacial acetic acid in sufficient H_2O to make 45 ml.

Dilute HCl.—Add sufficient H_2O to 15 ml conc. HCl to make 80 ml.

KI soln.—Dissolve 16.5 g KI in sufficient H_2O to make 100 ml.

DETERMINATION

Transfer the accurately weighed sample, equivalent to ca 0.25 gm quinacrine hydrochloride, to a 100 ml volumetric flask with the aid of 45 ml of the sodium acetate-acetic acid mixture. Shake thoroly to dissolve the quinacrine hydrochloride. Allow to settle and add exactly 50 ml 0.1*N* $K_2Cr_2O_7$. Fill to the mark with distilled water and mix well. Allow to stand 10–15 min and filter thru a dry filter, rejecting the first 15 ml of filtrate. Measure exactly 50 ml of the subsequent filtrate into a glass-stoppered flask. Add 80 ml of the dilute HCl and 20 ml of the KI soln. Stopper the flask and mix by gentle swirling. Allow to stand for 5 min. and titrate the liberated iodine with 0.1*N* $Na_2S_2O_3$ adding starch indicator when the end point is neared.

1 ml 0.1*N* $K_2Cr_2O_7$ is equivalent to 0.00842 g of $C_{23}H_{30}OClN_3 \cdot 2HCl \cdot 2H_2O$.

(4) The following method was adopted as official, first action.

DEMEROL

Distillation Method

APPARATUS

An all-glass apparatus was assembled, using pieces made with standard taper (24/40) ground glass points. It consisted of a 500 ml round bottom, short neck flask into which fitted an adapter with attached separatory funnel (Ace catalog, 5270). A distilling head (Scientific Glass Apparatus Co., J-1500) fitted into this adapter. A connecting adapter (Ace, 5125) was used to connect the distilling head to a straight inner-tube, water cooled condenser. Another adapter, attached to the bottom of the condenser, was long enough to dip below the surface of the liquid in a 1,000 ml Erlenmeyer flask. Other apparatus could be set up which would probably work equally as well. The essential features are provision for adding liquid during the distillation and a distillation head efficient enough to prevent any spray of the calcium carbonate mixture from carrying over into the receiving flask.

REAGENTS

Methyl red indicator. See 39.8(b).

Sodium hydroxide soln. 0.02 *N* NaOH standardized against sulfamic acid using methyl red indicator.

Sulfuric acid soln., 0.02 *N* H_2SO_4 standardized against the 0.02 *N* NaOH using methyl red indicator.

Powdered calcium carbonate.

PREPARATION OF SAMPLE

Weigh a counted number of not less than 20 tablets and reduce them to a fine powder without appreciable loss.

DETERMINATION

Weigh accurately an amount of the powder equivalent to ca 100 mg of Demerol. Wash it into a 500 ml round bottom flask with ca 25 ml of water. Introduce into flask ca 1 gm of powdered $CaCO_3$ and connect the flask to the distillation apparatus described above. Place 1-liter Erlenmeyer flask, containing 20 ml of 0.02 *N* H_2SO_4 under condenser in such manner that the adapter on end of condenser will be below surface of the acid. Thru separatory funnel introduce into flask 100 ml of water. Heat until only ca 25 ml of liquid remains in flask. Without interrupting distillation, add a second 100 ml of water slowly enough that distillate does not suck back into distillation flask. Continue distillation until this portion has distilled over. In like manner, distill over a third 100 ml of water. Then add, thru the separatory funnel, 10 ml of ethyl alcohol. When most of alcohol has distilled over, add and distill over a fourth 10 ml portion of water. Disconnect condenser from distillation apparatus and rinse inside of condenser and adapter which dipped into the standard acid, catching rinsings in a receiving flask. Bring collected distillate to a vigorous boil to remove any dissolved CO_2, cool, and titrate excess acid with 0.02 *N* NaOH, using methyl red as indicator. Each ml of 0.02 *N* H_2SO_4 = .005673 gm of demerol hydrochloride $C_{15}H_{21}O_2N \cdot HCl$.

Extraction Method

REAGENTS

See under Distillation Method.

PREPARATION OF SAMPLE

Same as for distillation method.

DETERMINATION

Weigh accurately a portion of powder, equivalent to ca 0.1 gm of demerol and macerate it with 10 ml of distilled water and 1 ml of normal H_2SO_4 for 2 hours. Decant liquid thru small filter into separatory funnel. Macerate residue with 5 ml of distilled water for 20 min, filter thru same filter and wash residue and filter with small portions of distilled water. Saturate the soln with NaCl, then add 5 ml of normal NaOH and extract with 25 ml of ether. Draw off aqueous layer into another separator, and repeat extraction of aqueous layer in similar manner 6 times, using 20 ml of ether each time. Wash combined ether extracts with two 5-ml portions of distilled water, then extract this water with 10 ml of ether and add this ether to main ether extract. Extract ether soln first with 20 ml of 0.02 *N* H_2SO_4, accurately measured, then successively with 10 ml and 5 ml of distilled water. Combine the H_2SO_4 and water extracts in beaker and warm on water bath until odor of ether is no longer perceptible. Cool soln and titrate excess acid with 0.02 *N* NaOH, using methyl red indicator. Each ml of 0.02 *N* H_2SO_4 is equivalent to .005673 gm of demerol hydrochloride, $C_{15}H_{21}O_2N \cdot HCl$.

(5) The following method was adopted as official, first action.

DIHYDROCODEINONE

Weigh a quantity of the powdered sample to contain ca 0.065 g of dihydrocodeinone. Transfer to a separator and dissolve in 15 ml of H_2O. Make alkaline to litmus with dilute NH_4OH. Extract with $CHCl_3$, using 30, 20, 20, and 10 ml portions. Filter into beaker and evaporate the combined $CHCl_3$ extracts on water bath to ca 5 ml, using a current of air to assist evaporation. Add 20 ml 0.02*N* H_2SO_4 and evaporate the remainder of the $CHCl_3$. Titrate the excess acid with 0.02 *N* NaOH, using methyl red as indicator. 1 ml of 0.02 *N* $H_2SO_4 = 0.00707$ g of dihydrocodeinone hydrochloride, $C_{18}H_{21}O_3N \cdot HCl \cdot H_2O$.

(6) The following method was adopted as official, first action.

THIOURACIL

Weigh an amount of sample equivalent to 0.25 gm (±0.040 gm) thiouracil and transfer to a 100 ml volumetric flask. Add ca 50 ml of distilled water and then add 3 ml of 5% NaOH. Shake for a few min. Dilute to volume with distilled water, shake, filter thru a dry filter, and transfer 50 ml of the filtrate to an iodine flask. Add 25 ml of 0.5 *N* standard KBr-$KBrO_3$ and 10 ml of 10% hydrochloric acid, and let stand for exactly 15 min. Then add 10 ml of 15% KI soln and let stand for exactly 5 min. Titrate the iodine with 0.1 *N* $Na_2S_2O_3$ and take the first disappearance of the yellow (or blue) color as the end point. The use of starch as an indicator in this titration is optional. One ml of 0.1 *N* KBr-$KBrO_3$ is equivalent to 0.001282 g of thiouracil ($C_4H_4ON_2S$).

(7) The following method was adopted as official, first action.

OINTMENT OF BENZOIC AND SALICYLIC ACIDS

Transfer an accurately weighed sample, ca 2.5 g, into a separatory funnel, add ca 50 ml of ether and swirl until dissolved. Completely extract with saturated soln of $NaHCO_3$, using 15, 15, 10, and 10 ml portions, or more. Extract the combined $NaHCO_3$ soln with 10 ml of $CHCl_3$ and discard the latter. Acidify with HCl and extract with $CHCl_3$ ether (2+1) until the benzoic and salicylic acids are completely extracted. Filter extracts into a 250 ml beaker thru filter moistened with $CHCl_3$. Evaporate to ca 5 ml on a steam bath using a current of air, then continue spontaneously.

Dissolve residue in about 20 ml of diluted alcohol (about 50%), carefully titrate with 0.1 *N* NaOH using phenolphthalein, record the volume, and add an excess of ca 2 ml. Completely evaporate the alcohol on a steam bath using a current of air; evaporation from ca 50 ml volume to 5 or 10 ml is sufficient. (Alcohol consumes bromine.)

Transfer remaining titration liquid and washings to 100 ml volumetric flask, cool to room temperature, and fill to mark with water. Mix thoroly. Pipet a 25 ml aliquot into an iodine flask, add 25 ml of water, exactly 25 ml of 0.1 *N* KBr-$KBrO_3$, and ca 5 ml of HCl. Swirl mixture repeatedly during 30 min. Carefully add 5 ml of KI soln (ca 10%), shake well and in about one min. titrate with 0.1 *N* $Na_2S_2O_3$ using starch indicator.

Calculate salicylic acid from the 0.1 *N* KBr-$KBrO_3$ consumed, 1 ml = 0.0023 g. Calculate benzoic acid from the difference between the 0.1 *N* NaOH titration value and the 0.1 *N* NaOH equivalent of the salicylic acid found in sample taken. 1 ml of 0.1 *N* NaOH = 0.01221 gram of benzoic acid or 0.01381 gram of salicylic acid.

40. MICROBIOLOGICAL METHODS

No additions, deletions, or other changes.

41. MICROCHEMICAL METHODS

No additions, deletions, or other changes.

42. EXTRANEOUS MATERIALS IN FOODS AND DRUGS

(1) The tentative method for rodent excreta in corn meal, section **42.32** (p. 781), was made official, first action.

(2) The official method for molds in tomato products, section **42.57** (p. 788), was changed, official, final action, as follows:

(a) Deleting beginning "mix," line 3, par. 1, to end of paragraph.

(b) Substituting "add H_2O to make a mix having a total solid content such as will give an immersion refractometer reading at 20° of 45.0–48.7 or an Abbé refractometer reading at 20° of 1.3447–1.3460."

(c) Introducing a reference in the selected references, Chapter 42, applying to this change, as follows: "National Canners' Assoc. Bul., 27–1, revised Feb. 1941, Table 3, p. 58."

(3) The tentative method for manure fragments in dairy products, section **42.11** (p. 774), to be changed by substituting "up to" for "ca" in line 3.

(4) The word "examination" in line 1 of the method for dried mushrooms, *This Journal*, **30**, p. 105, was changed to "contamination."

43. STANDARD SOLUTIONS

(1) The official, first action, method for sodium thiosulphate, sections **43.28–43.29** (p. 809), was adopted as official, final action.

(2) The official method for standardization of potassium permanganate, section **43.18** (p. 807), was deleted, first action.

(3) The following method for standardization of potassium permanganate was adopted as official, first action.

STANDARDIZATION

Transfer 0.3 g of dried (105°) sodium oxalate (National Bureau of Standards standard sample) to a 600-ml beaker. Add 250 ml of diluted sulfuric acid (5+95) previously boiled for 10–15 min and then cooled to 27° ± 3°.

Stir until the oxalate has dissolved. Add 39 to 40 ml of 0.1*N* potassium permanganate at a rate of 25 to 35 ml per minute while stirring slowly. Let stand (about 45 seconds) until the pink color disappears. If the pink color should persist because the permanganate is too strong, discard, and begin again, adding a few ml less of the $KMnO_4$ soln. Heat to 55 to 60°C, and complete the titration by adding permanganate until a faint pink color persists for 30 seconds. Add the last 0.5 to 1 ml dropwise with particular care to allow each drop to become decolorized before the next is introduced.

Determine the excess of permanganate required to impart a pink color to the solution. This can be done by matching the color by adding permanganate to the same volume of the boiled and cooled diluted sulphuric acid at 55 to 60°C. This correction usually amounts to 0.03 to 0.05 ml.

(4) Add the following reference in selected references, Chapter 43, as applying to method in (3): *J. Research Natl. Bur. Standards,* **15,** 493 (1935), Research Paper No. 843.

(5) In the method for thiocyanate, paragraph on reagents (*This Journal,* **30,** 105, 1947), the reference (4) is as follows: (4) See Volumetric Analysis, Kolthoff and Furman, Vol. II (1929).

(6) The following method was adopted as official, first action.

BROMIDE—BROMATE

REAGENTS

Bromide-bromate soln.—Weigh out about 2.8 grams of potassium bromate and 12 grams of potassium bromide into a one liter flask. Fill with boiled distilled water.

Arsenious oxide soln.—Prepare standard arsenious oxide by the method given in **43.19** and **43.20.**

STANDARDIZATION

Measure 40 ml of the standard arsenite soln from a buret into a 300 ml Erlenmeyer flask. Add 10 ml HCl and 3 drops of methyl orange (**43.12(a)**). Titrate with the standard bromide-bromate solution until one drop, or less, causes the color of the methyl orange to fade completely. The soln should be swirled constantly and the last ml added dropwise with swirling between each drop.

(7) The method for thiocyanate soln, *This Journal,* **30,** 105 (1946), was adopted as official, final action.

ERRATA AND EMENDATIONS, METHODS OF ANALYSIS, A.O.A.C. 1945

Supplemental to list as published, *This Journal,* 30, 60 (1947)

Section	*Page*		
15.5	182		Raise section number, **15.5,** to aline with "Glycerol in dry wines." In same paragraph change "allowering" to "allowing."
16.3	191	line 1	Change "HO_2" to "H_2O."
19.16(a)	227	line 7, par. 2	Change "ca 20° (below 25°)" to "20° or lower."
20.125	268	3d line from end	Change "1 ml" to "0.1 ml."
27.59	419	line 11	Insert the words "Cool, make to measured volume, and mix." before "Compare."
	521	Reference 25	Delete "82."
42.62	790	Title .1	Insert reference (6) before (7).

ANNOUNCEMENTS

The 62d Annual Convention of the Association of Official Agricultural Chemists will be held October 11 to 13, inclusive, at the Shoreham Hotel, 2500 Calvert Street, N. W., Washington 8, D. C.

As the Shoreham Hotel will provide the halls and other facilities for the meeting it is urged that members and visitors secure their accommodations at this hotel if possible and make it their headquarters. Twin bed double rooms are $8.00 and $9.00 per day. A limited number of single rooms may be available at $6.00 and $7.00. All rooms are with private bath. The rates are on the European plan. It is suggested that reservations be secured well in advance of the meeting, by direct communication with the hotel.

NOTICE TO SUBSCRIBERS

The Association urgently needs a substantial number of copies of Vol. 29, No. 1, the Feb. 15, 1946, issue of its *Journal*; and, in addition, all four numbers, Vols. XXIV and XXV; Vol. XXVII, No. 4 (November, 1944); and Vol. 28, No. 2 (May, 1945). Those having copies not intended as permanent files will favor the Association by returning them. Payment of $1.60 per copy will be refunded to cover their cost and postage as second class matter.

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CORRECTIONS—NOVEMBER JOURNAL

In the paper on "Factors Affecting the Availability of Ammoniated Superphosphates. Part II," by William H. Ross, J. Richard Adams, John O. Hardesty, and Colin W. Whittaker, published in the preceding number of *This Journal*, **30**, 624 (1947), in Table 10, p. 634, last figure in the last column should read "−27" instead of "+27."

CONTRIBUTED PAPERS

THE USE OF THE SODIUM-CHLORINE RELATIONSHIP FOR THE DETECTION OF SODIUM NEUTRALIZED NON-FAT DRY MILK SOLIDS

By WILLIAM HORWITZ (Food and Drug Administration, Federal Security Agency, Minneapolis, Minnesota)

The presence of neutralizers in non-fat dry milk solids can be detected by the alkalinity of ash method as developed by Hillig (1). The reliability of this method is supported by the work of Kunkel and Combs (2) and it has been adopted as official by the A.O.A.C. (3). The chief disadvantage of this procedure is a lack of precision since a large excess of acid must be added to the ash and 95 per cent of it is back titrated to arrive at the alkalinity value.

The use of the mineral constituents of milk ash as an index of neutralization is mentioned by Davies (4). The phosphorus content of milk is reported to be a straight line function of the calcium content, and, likewise, the sodium content is a straight line function of the chlorine content. The purpose of this work was to obtain additional data to support the use of the sodium:chlorine ratio (5) as an index of sodium neutralization in non-fat dry milk solids.

METHODS

The methods used in this study were:

Ash.—Methods of Analysis, section 22.99.

Alkalinity of Ash.—Methods of Analysis, section 22.100.

Chlorine.—Methods of Analysis, section 23.22. Open Carius on 2 g sample, using 10 ml 0.1*N* $AgNO_3$ and back titration with 0.05*N* thiocyanate.

Sodium —Methods of Analysis, section 12.21. Magnesium uranyl acetate on the sulfated ash from 2 g sample. Solution made up to 100 ml after precipitation of phosphates, filtered, and 50 ml aliquot used for the determination. It was later determined that the use of the sulfated ash was unnecessary since the direct ash (*Methods of Analysis*, 22.99) gave similar results.

SAMPLES

A total of 104 samples divided into three groups were examined for sodium and chlorine in this study. The first group consisted of 15 samples of authentic dry milks prepared by Hillig in 1941 (1). Nine of these samples were unneutralized; six were neutralized. The second group consisted of 58 samples which had previously been examined for neutralization in this laboratory. Fifteen of these samples were considered neutralized by the alkalinity of ash determination. The Kunkel-Combs criterion of the alkalinity of the ash of 130 ml 0.1*N* HCl/100 g sample was used as the base line in this study for determining whether a given sample was to be classified as neutralized. The third group consisted of 31 samples of unknown history which were collected for this study from ordinary commercial channels. Two samples of this group showed an abnormal sodium to chlorine ratio and the presence of a neutralizer was confirmed by alkalinity of ash determinations. The other 29 samples of this group were not examined for alkalinity of ash and, for brevity, they will be referred to as normal or as unneutralized, with the understanding that they are normal only with respect to sodium neutralization and that the possibility that some of these samples may have been neutralized with other than sodium compounds can not be ruled out.

RESULTS

Figure 1 shows the individual values plotted with per cent sodium as the abscissa and per cent chlorine as the ordinate. All of the data are expressed

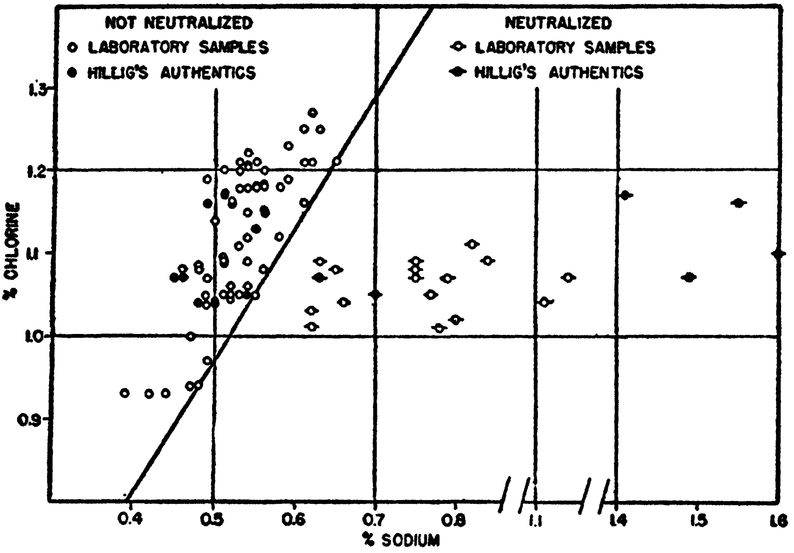

FIG. 1.—The sodium and chlorine contents of 104 non-fat dry milk solids samples. The sodium axis is omitted from 0.86 to 1.08 per cent sodium and from 1.16 to 1.38 per cent sodium without omitting any data or changing the scale.

on an *as is* basis, since the ratio of the two components is independent of the method of expression of the results. For convenience, the sodium axis has been broken at two points without eliminating any data or changing the scale. The circles represent normal samples with an alkalinity of ash value of less than 130. The solid circles represent the values obtained on the authentic unneutralized samples prepared by Hillig. A similar representation is used for the neutralized authentic material prepared by Hillig and the neutralized commercial samples, *i.e.*, having an alkalinity of ash greater than 130. The average sodium to chlorine ratio of the 81 unneutralized samples is 0.47 with a standard deviation of 0.03. The maximum ratio is 0.54. The minumim ratio of the neutralized samples studied is 0.58.

The equation of the line which marks the high sodium boundary of the normal samples in figure 1 is:

$$\% \text{ Na} = 0.62\% \text{ Cl} - 0.10.$$

This equation may be used instead of the ratio sodium:chlorine to determine the presence of a sodium neutralizer. If the per cent chlorine in the sample (*as is* basis) is substituted into the equation, the calculated per cent sodium should be equal to or greater than the actual sodium content of the sample as determined by analysis if the sample is to be classified as normal (or non-sodium neutralized). If the actual sodium content is greater than the calculated sodium content, then the dry milk was prepared from a sodium neutralized skim milk.

This method of approach to the problem of detecting the presence of neutralizers can easily be extended to calcium neutralized dry milks, if encountered, since the ratio of calcium to phosphorus is 1.3 (6). The magnesium content of normal milk is so low (7) that a direct analysis for this constituent would be sufficient to establish its presence in excessive quantities.

The value of this method of detecting neutralized dry skim milk lies in the fact that it is based upon definite chemical constants which can be determined with a relatively high degree of accuracy and precision as compared with the empirical alkalinity of ash determination. It has the disadvantage that the time involved is considerably greater than that required for the alkalinity determination.

SUMMARY

The sodium and chlorine contents of (1) 23 sodium neutralized and (2) 81 normal or non-sodium neutralized dry skim milks were determined. The ratio of sodium to chlorine in the normal samples was 0.47 with a standard deviation of 0.03. If the per cent sodium is plotted as the abscissa against the per cent chlorine as the ordinate, all of the sodium neutralized samples lie to the right of the line represented by the equation: $\% \text{ Na} = 0.62\% \text{ Cl} - 0.10$. All of the normal or non-sodium neutralized samples lie to the left of this line.

ACKNOWLEDGEMENT

The assistance of Dona S. Clark is gratefully acknowledged.

BIBLIOGRAPHY

(1) Hillig, F., *This Journal*, **22**, 495 (1939); **23**, 468 (1940); **24**, 556, 744 (1941); **25**, 253, 610 (1942); **28**, 205 (1945).

(2) Kunkel, R. W., and Combs, W. B., *J. Dairy Sci.*, **28**, 219 (1945).

(3) A.O.A.C., *Methods of Analysis*, 6th Ed., 22.99, 100.

(4) Davies, W. L., *J. Soc. Chem. Ind.*, **57**, 246 (1938).

(5) Jones, T. S. G., and Davies, W. L., *Biochem. J.*, **29**, 978 (1935).

(6) Bulletin 905, Revised 1946, American Dry Milk Institute, Inc., 221 North LaSalle Street, Chicago 1, Illinois, page 5.

(7) Davies., W. L., "The Chemistry of Milk," D. Van Nostrand Co., New York, Second Edition, 1939.

SERUM METHODS FOR ADDED WATER IN MILK

By D. J. Mitchell and Guy G. Frary, State Chemical Laboratory, Vermillion, So. Dak.

The Association of Food, Dairy, and Health Officials of the South Central States at a recent conference adopted a resolution that a study be made of the official method for added water in milk involving the sour serum (*Methods of Analysis*, 6th Edition, **22.29**). The Association found need for further information as to the reliability of this method for detecting added water in amounts of less than thirteen per cent. Since so wide a departure from accuracy as reported by the South Central officials, if true for any considerable percentage of samples of normal milk, would effect a most generous tolerance for adulteration, it was thought a review of this and allied methods might be in order.

Our *Methods of Analysis*, Sixth Edition, describes three methods (**22.28**, **22.29**, and **22.30**) by which milk serum may be prepared for examination by means of the immersion refractometer. The first two of the methods also call for determination of ash in the serum as an aid in detection of added water. Leach and Lythgoe[1] made a study of the acetic serum method. Their report on thirty-two separate samples collected and examined in the routine of inspection gave the maximum immersion refractometer reading on the serum of 44.5, the minimum as 34.7, with an average of 42.1. From the determinations of total solids, fat, solids not fat and ash, the sample with the reading 34.7 appeared to be watered. Two samples had a refractometer reading below 39.0, which is the minimum reading for acetic serum obtained from normal milk, according to the present official method (**22.28**). Readings between 39.0 and 40.0 are suspicious and ash is to be determined on the serum when the reading is 40.0 or below. Only three samples in the group studied by Leach and Lythgoe gave a reading below 40.0. They also reported on twenty-two individual fancy Holstein and twelve individual common Holstein cows. The maximum immersion refractometer reading on the serum of the fancy cows was 44.5, the minimum 40.0, with an average of 42.7. The maximum immersion refractometer reading on the serum of the common cows was

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[1] *J. Am. Chem. Soc.*, **26**, 1195 (1904).

43.1, the minimum 39.0, with an average of 41.6. Only three samples had a refractive index below 40.0.

Lewis I. Nurenberg[2] made a study of the refractometer readings obtained using the sour serum method and also on the amount of ash present in both the acetic and sour serum. In his report on 660 individual cows, there was not any refractometer reading below 38.3, which is the minimum reading for serum obtained from normal milk by means of simple souring, according to the present official method **(22.29)**. Only 5.2 per cent of the samples gave readings below 39.0. The refractometer readings ranged from 40.0 to 43.9 on 77.3 per cent of the samples. Nurenberg's report on 49 separate herds did not show any refractometer readings below 39.0 and only 4.1 per cent below 40.0. In his report on the ash obtained on the sour serum from the milk of 504 individual cows, there were none showing an ash below 0.730 gm/100 ml of serum, which is the minimum for normal milk, according to the present official sour serum method **(22.29)** and only 8.2 per cent below 0.740 gm/100 ml of serum. From 35 separate herds not any of the samples had a sour serum ash below 0.740 gm/100 ml, and only 3 samples (8.6%) were below 0.760 gm/100 ml. Nurenberg also published the results submitted by Leslie W. Ferris of the U. S. Bureau of Chemistry, who made a comparison of ash values obtained on the acetic serum and the sour serum of 40 herd samples. The maximum for the acetic serum ash was 0.877 gm/100 ml of serum, the minimum was 0.709 gm/100 ml, with an average of 0.769 gm/100 ml. Only 7 samples gave an ash below 0.715 gm/100 ml, which is the minimum for normal milk, according to the present official acetic serum method **(22.28)**. For the sour serum method, the maximum ash was 0.924 gm/100 ml, the minimum 0.671 gm/100 ml (later raised to 0.722 gm/100 ml upon further souring), with an average of 0.765 gm/100 ml. Nuremberg recommended the adoption of the current sour serum method and also the adoption of the minimum values for ash used in both the present acetic and sour serum methods.

Since it appeared that the sour serum method did not give accurate results, this brought suspicion on the other methods, so the three official serum methods, 22.28, 22.29 and 22.30, were included in this preliminary qualitative investigation. The sour serum method was modified by adding about 1 per cent (20 drops to 100 ml of milk) of buttermilk culture to aid the souring. It was determined that 1 per cent of culture was as effective as 2 per cent and that it was necessary to let the milk sour at least 40 hours at near 30°C for satisfactory results. Twenty-seven herd samples of raw milk were examined. Each sample was divided into five parts and systematic dilutions of 0, 5, 10, 15, and 20 per cent of water were used. The cryoscopic method was used only on the undiluted samples. The maximum, minimum, and mean values are given in Table 1.

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[2] *This Journal*, **2**, 145 (1916).

An inspection of the minimum values in the above table shows that in the case of all three methods, more than 5 per cent of added water must be present before it would be indicated, if one accepts as correct the immersion refractometer readings given in the present methods. The minimum value for the acetic serum at 5 % dilution arouses suspicion. Five samples of the acetic serum at 5% dilution gave readings between 39.0 and 40.0. The mean values for all three serum methods do not indicate added water until more than 10 per cent is present.

Eight samples of the acetic serum at 10% dilution gave readings below 39.0. Five samples of the sour serum at 10% dilution gave readings below 38.3 and four samples of the copper serum at 10% dilution gave readings below 36.0.

TABLE 1.—*A study of 27 herd samples of milk*

% ADDED WATER		IMMERSION REFRACTOMETER READING AT 20°C.			ASH GMS/100 ML OF SERUM		TRUE FREEZING POINT
		ACETIC SERUM	SOUR SERUM	COPPER SERUM	ACETIC SERUM	SOUR SERUM	
None	Max.	44.1	43.5	39.2	0.8324	0.8916	0.561
	Min.	40.3	40.0	37.0	0.7104	0.7608	0.530
	Mean	42.5	41.8	38.3	0.7751	0.8001	0.544
5	Max.	42.5	41.9	38.2	0.7940	0.8384	
	Min.	39.0	38.3	36.1	0.6732	0.7040	
	Mean	41.0	40.4	37.3	0.7352	0.7557	
10	Max.	41.0	40.5	37.1	0.7540	0.7916	
	Min.	37.8	37.5	35.1	0.6372	0.6536	
	Mean	39.6	39.0	36.3	0.6995	0.7172	
15	Max.	39.5	38.9	35.9	0.7020	0.7428	
	Min.	36.5	36.2	34.1	0.5920	0.6260	
	Mean	38.1	37.5	35.3	0.6584	0.6748	
20	Max.	37.8	37.5	35.0	0.6568	0.6984	
	Min.	35.1	34.9	33.3	0.5816	0.6052	
	Mean	36.7	36.1	34.3	0.6200	0.6362	

This minimum value from Table 1 for the acetic serum ash in the undiluted milk is below the value given in the method, but the freezing point of the sample does not indicate added water. The mean values for the ash does not indicate added water until more than 5 per cent is present in both the acetic and sour serum methods. Three samples of the acetic serum at 5% dilution gave an ash below 0.715 gm/100 ml. Three samples of the sour serum at 5% dilution gave an ash below 0.730 gm/100 ml.

From the early work it appeared that it was the rule to use the lowest figure that had been obtained for the immersion refractometer reading and the ash as the criteria in determining if added water was present. Using this as a basis, it would be possible for a milk with a fairly high refractive index and ash to contain at least 10 per cent added water before it could be detected.

It is believed that the lower limits for the immersion refractometer reading and for minimum serum ash in the present Official Methods should be raised. The sour serum method is objectionable for two reasons. First, it is impossible for pasteurized milk to sour spontaneously, and second, the statement "completely sour" is quite indefinite. If the milk is partially sour, the ash will probably be low because some of the calcium is precipitated with the casein[3] and not redissolved until the acidity approaches a maximum.

The copper serum method is the most rapid of the three from the standpoint of preparation of the serum and is desirable since it gives a narrow range of readings.

The cryoscopic method should be used to check the serum methods whenever added water is indicated. This is a rapid, accurate, and most reliable method.

STIRRER ACTUATED CONTINUOUS ETHER EXTRACTOR

By Morris L. Yakowitz and Herman J. Meuron

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A continuous extractor is described which employs a novel stirrer-pump to force the extracting solvent through the sample and absorbing solutions. The device has a rapid rate of flow and can be used with large or small volumes. The process is carried out at room temperature and the extracted material is not subjected to heat.

In removing an extractable substance from a solution by means of an immiscible solvent, the commonly used continuous extractor is that of Palkin (1) or Widmark (3), or a modification of one of these.

In the Palkin type continuous extractor, the extracted substance collects in a distilling flask from which the solvent is continuously distilled back into the vessel containing the sample. The extracted substance is subjected to heat at the boiling point of the solvent, and mixed solvents of greatly different volatilities cannot be used.

In the Widmark type continuous extractor, the solvent is in contact with the sample solution and an absorbing solution which changes the composition of the extracted substance so that it is no longer soluble in the solvent. The extracted substance collects in the absorbing solution, and the extraction process can be carried out at any desired temperature including room temperature. However, the original Widmark apparatus (3) and the Pucher and Vickery modification (2) are slow.

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[3] Nurenberg, Lewis I., *Loc. cit.*

The authors have devised an extractor like that of Widmark, in which the solvent is circulated from the compartment containing the sample solution, into a compartment containing the absorbing solution. In the authors' extractor, the movement of the solvent is produced by a novel stirrer-pump which gives a rapid rate of flow and causes the solvent actually to flow through both the sample solution and absorbing solution, resulting in a rapid rate of extraction.

The principle of the stirrer actuated extractor is shown in Figure 1, and a recommended model with dimensions is shown in Figure 2.

PRINCIPLE OF EXTRACTOR

In Figure 1, the left-hand vessel is divided into two compartments A and B, separated by a partition which has a hole at its center. A stirrer with vertical blades rotates just above the partition. The rotating stirrer causes the liquid in A to swirl and gives the liquid a deeply concave surface. This creates a hydrostatic head of pressure between the outlet on the sidewall and the inlet hole at the center of the partition. If the liquid in A is swirled rapidly enough to bring the concave surface down tangent to the partition, this external difference in pressure is equal to the height which the swirling liquid reaches on the sidewall, measured from the level of the partition. Actually, this pressure head is increased about 10–20 per cent by the centrifugal throw of the swirling liquid at the outlet hole. The maximum driving pressure which can be obtained with the stirrer pump is determined by the height of compartment A.

To operate, a suitable volume of sample solution is placed in compartment C to the level shown, the absorbing solution is placed in compartment B, and solvent is added until it has filled C to above its outlet and A is about half-filled. The stirrer is set in motion and when the swirling of liquid in A is great enough, solvent will flow out the side-tube from A, rise through sample solution in C, thence through absorbing solution in B, and back into the stirrer chamber, A.

If the sample solution in C is made alkaline while the absorbing solution in B is acidic, alkaline substances such as alkaloids will be removed from the sample solution and will collect in the absorbing solution. To extract organic acids from the sample solution it is necessary merely to reverse these conditions.

DESIGN OF PRACTICAL EXTRACTOR

The design and operation of the extractor shown in Figure 2 is based on the following practical considerations.

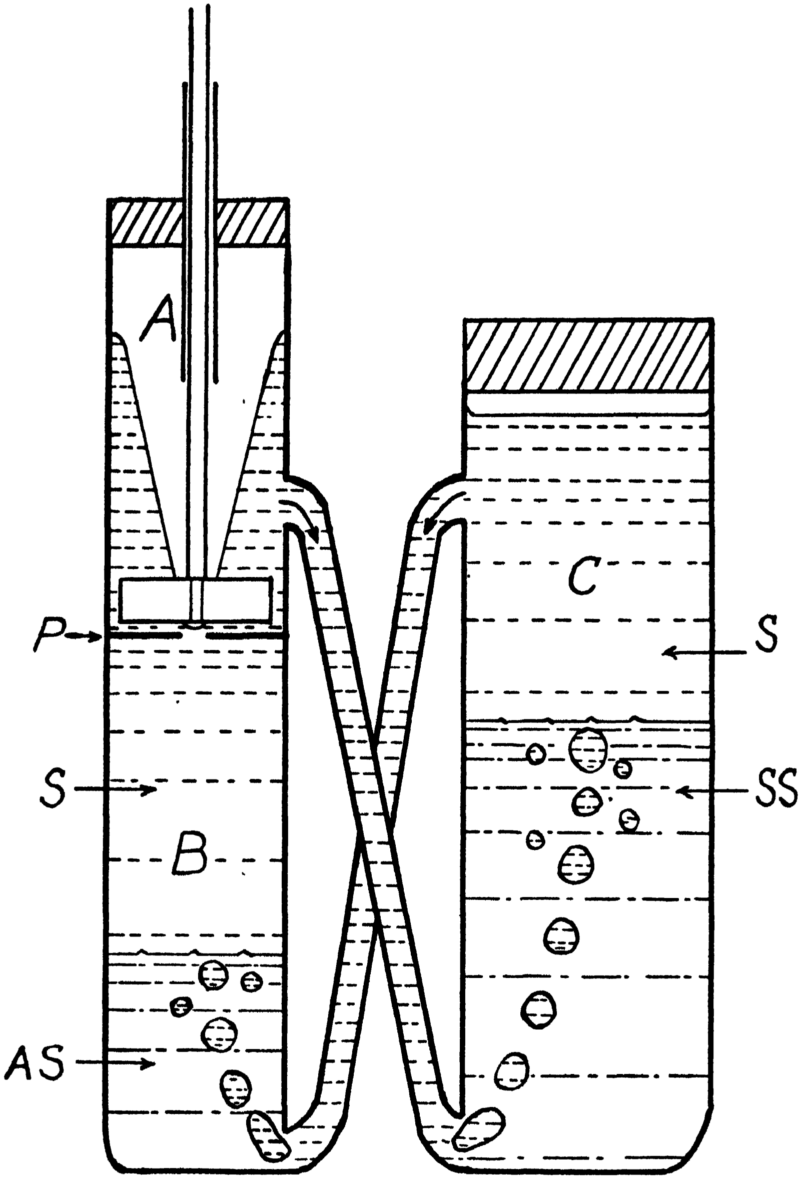

FIG. 1. Principle of Operation
A. Stirrer-pump compartment
B. Absorbing solution compartment
C. Sample solution compartment
P. Partition
S. Solvent
AS. Absorbing Solution
SS. Sample Solution

The pump must overcome the resisting hydrostatic head of pressure due to the difference in density of solvent and aqueous layers, and must also provide enough additional pressure to create a rapid rate of flow of solvent through the connecting tubes. The first resistance is a function of the differences in density between solvent and aqueous column. Thus, to force ether through 10 inches of water, the pump must furnish a minumim head of 4 inches of pressure before any ether will flow. In the apparatus shown in Figure 2, which has a total volume of 500 ml, the pump chamber has a height of 7 inches which allows it to force ether quite rapidly through a combined aqueous layer of up to 10 inches.

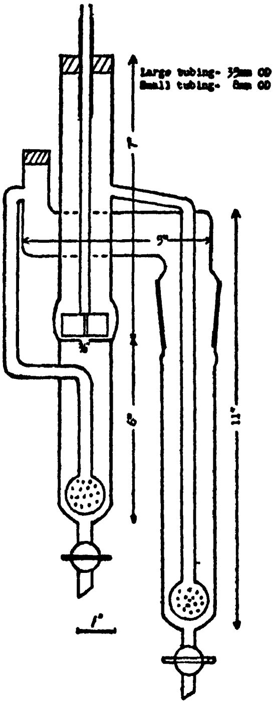

FIG. 2. Recommended Analytical Extractor

The solvent should pass through the aqueous layers in small droplets in order to achieve rapid exchange of dissolved matter, so some form of distributor is desirable where the solvent enters the aqueous portions. A satisfactory distributor may be made by blowing a small glass bulb and then blowing small holes in this bulb. Sintered glass discs offer too much resistance to the flow of liquid and are not satisfactory. A much better distributor can be made by sealing a porcelain Witte plate into Pyrex tubing, bent so that the droplets escape directly up into the solution. For solvents like benzene, whose droplets tend to coalesce when rising through water, a platinum filter cone may be sealed into Pyrex tubing in place of the Witte plate.

There is a tendency for emulsions to form, so there must be a volume of solvent above both sample and absorbing solution to permit the breaking of such emulsions.

The outlet from the stirrer chamber should be about half way up the side of the chamber and approximately at the same level as outlet tube from vessel containing sample. This makes it possible to fill the sample vessel right up to its stopper when the stirrer chamber is half full, and yet allows emptying of the absorbing compartment without siphoning sample solution over.

TABLE 1.—*Experimental results*

SAMPLE SOLUTION	ABSORBING SOLUTION	EXTRACTION TIME	RECOVERY
			Per cent
.1240 gm ephedrine hydrochloride in 100 ml of 1% NaOH, 1% NaCl solution.	25 ml of .2N · HCl	20 Min.	98.5
.1587 gm ephedrine hydrochloride in 150 ml of 1% NaOH, 1% NaCl solution.	25 ml of .2N · HCl	20 Min.	99.0
.1005 gm sodium oxalate in 100 ml of .2N · H_2SO_4.	25 ml of 2% $NaHCO_3$	90 Min.	99.0
.1005 gm sodium oxalate in 100 ml of .1N · H_2SO_4.	25 ml of 2% $NaHCO_3$	105 Min.	99
.129 gm citric acid in 150 ml of .1N · H_2SO_4.	40 ml of 2% $NaHCO_3$	2 Hours	25
.129 gm citric acid in 150 ml of .1N · H_2SO_4	40 ml of 2% $NaHCO_3$	4 Hours	40
.025 gm monochloracetic acid in 175 ml of 1N · H_2SO_4	30 ml of 1N NaOH	15 Min.	95
.025 gm monochloracetic-acid in 175 ml of 1N · H_2SO_4.	30 ml of 1N NaOH	15 Min.	95

The stirrrer chamber should be expanded into a slight bulge just above the partition which separates it from the absorbing compartment. This permits the four-bladed stirrer to rotate freely without striking the sidewall.

The apparatus is made in two pieces with a ground glass joint to permit use of various sizes of sample solution containers, and also to permit ease in cleaning apparatus. Each compartment is provided with a stopcock to facilitate emptying.

The connecting tubing is made of relatively large diameter to decrease resistance to flow and at the same time strengthen the apparatus.

The stirrer may be turned by any suitable device. A simple air-turbine, and a small, rheostat-operated fan motor have both been used. The cork stopper through which the bearing sleeve passes should have a small hole cut through it so that atmospheric pressure is maintained in the stirrer chamber. The well-fitting bearing may be lubricated with a few drops of heavy mineral oil.

EXPERIMENTAL

The apparatus was used to extract ephedrine, oxalic acid, citric acid, and monochloracetic acid, washed ether being employed as the solvent in each case. Results are shown in Table 1. In making these extractions, a stirrer speed of about 1500 r.p.m. was used, giving a flow of about 200 ml of solvent per minute.

LARGE VOLUME EXTRACTOR

The apparatus shown in Figure 3 was made to extract from a large container such as a 20 liter bottle. The stirrer compartment, absorbing solution compartment, and connecting tubing are separate pieces. The dropping funnel in the bottle is added to facilitate charging. This apparatus was used by the authors in making extractions from a 23 liter bottle (20 liters sample solution). The motion of the solvent droplets through the sample solution is usually enough to keep it well agitated.

A variation of this apparatus may be used to extract from a large open cylinder. The tubing leading from the stirrer chamber into the cylinder is bent to form an inverted U, allowing the solvent in the cylinder and and stirrer chamber to be at the same level.

Emulsions often form and the usual methods of breaking them should be tried. It has been our experience that a small motor or air-driven stirrer placed just above the sample solution, in the solvent, will break certain types of emulsions. If particles of emulsion are unavoidably carried into the absorbing solution, a further extraction from the absorbing solution (made acidic or basic as required) can be made using a smaller extractor, after the extraction from the original sample has been completed.

Using 20 liters of acidified water containing 22.9 mg of monochloracetic acid, 54.5% of the chloracetic acid was extracted after 2 hours operation. Using 2 liters of beer to which was added 176 mg monochloracetic acid, recovery was 100% after 1 hour of operation. The rate of circulation was approximately 500 ml of solvent per minute.

DISCUSSION

The apparatus as described has been tested with ether as the solvent and has been found to be a useful analytical tool. The large scale extractor should find use in extracting substances of biological origin which occur in small concentrations.

Experiments with various solvents have shown that there should exist a considerable difference in specific gravity, approximately .3 unit, between solvent and sample solution so that the two liquids may separate cleanly before the solvent enters the next compartment.

A considerable difficulty encountered is due to the formation of troublesome emulsions which tend to separate slowly. Each such case presents its own problem and various expedients must be tried. A fine "spray" of sample solution droplets often forms in the solvent phase and is carried around by the circulating solvent, but ordinarily the effect of this is negligible.

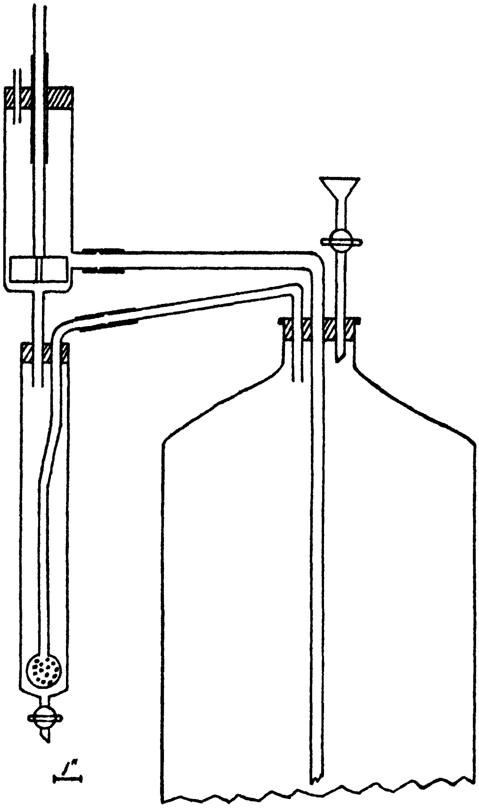

FIG. 3. Extraction Apparatus for Large Volumes

Large variations in stirring speed during operations are undesirable and should be prevented.

The stirrer pump may be employed in a continuous extractor using solvents heavier than the sample solution, and the authors hope to describe such an extractor at a future date.

LITERATURE CITED

(1) PALKIN, S., MURRAY, A. G., and WATKINS, H. R., *Ind. Eng. Chem.*, **17**, 612 (1925).

(2) PUCHER, G. W., and VICKERY, H. B., *Ind. Eng. Chem., Anal. Ed.*, **11**, 656 (1939).

(3) WIDMARK, E. M. P., *Skand, Arch. Physiol.*, **48**, 61 (1926).

THE DETERMINATION AND IDENTIFICATION OF LACTIC AND SUCCINIC ACIDS IN FOODS*

By H. V. CLABORN† and W. I. PATTERSON (Food & Drug Administration, Federal Security Agency, Washington 25, D. C.)‡

Numerous methods have been described for the determination of lactic acid, but practically all are not strictly specific, being based upon either the conversion to acetaldehyde (1, 2, 3, 4) or the color reaction with ferric chloride (5, 6). Previous methods for determining succinic acid in foods have depended for the most part upon ether extraction, followed by purification of the succinic acid by oxidation of contaminants (7). More recently, some excellent enzymatic methods have appeared (8, 9). Some of the above methods for lactic and succinic acids are very accurate and reliable for most purposes, but methods are needed by which these acids may be determined and *identified*, and that is the purpose for which the methods described below were developed. They furnish a means by which lactic and succinic acids may be quantitatively isolated and definitely identified in each sample analyzed.

The excellent specificity of the technique termed partition chromatography has been demonstrated in the analysis of the lower volatile fatty acids (10). The desirability of extending a method with such specificity to the separation of other acids which occur in food products, is obvious. A procedure utilizing this principle for separating a variety of polycarboxylic acids, including succinic acid, has already been used (11). Dilute sulfuric acid was used for the immobile solvent on silica gel and n-butanol-chloroform for the mobile solvent. Such a column required the use of an external indicator. Our experience, however, has demonstrated the convenience of an internal indicator on the partition column.

The method described below consists of five steps: (a) ether extraction (b) precipitation of the barium succinate from 80% alcohol, (c) isolation of lactic acid from the filtrate of (b), and of succinic acid from the insoluble fraction of (b) by partition chromatography, (d) titration of the acids with barium hydroxide, and (e) identification of the acids by microscopic examination of the barium salt of succinic acid or the zinc salt of lactic acid.

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‡ Contribution from the Food Division, W. B. White, Chief.

With modifications in the preparation of the sample, and separation of the acids from the sample in a form suitable for passage through the column, these procedures should be applicable to any food product; however, the procedures below are given for the determination of lactic acid in liquid or dried milks, and for the determination of succinic acid in liquid, frozen, or dried eggs.

METHODS

APPARATUS

Continuous extractor.—*Methods of Analysis, A.O.A.C.*, 1945, sec. 22.8 (p. 304).

REAGENTS

(a) *Solvent.*—20% tertiary butanol in chloroform V/V.

(b) *Glycerol indicator soln.*—150 mg of Alphamine Red-R dissolved in 100 ml of U.S.P. glycerol. This indicator is the ammonium salt of 3, 6 disulfo-beta-naphthalene-azo-N-phenyl-alpha-naphthylamine, also called R–NH_4 indicator, and is prepared according to Liddell and Rydon (12).

(c) *Dilute sulfuric acid* (1+1).

(d) *Concentrated sodium hydroxide soln.*—ca 40%.

(e) *Standard barium hydroxide soln.*—0.05 *N*.

(f) *Phenolphthalein indicator.*—1% in alcohol.

(g) *Silicic acid*[1]

(h) *Ammonium hydroxide.*—ca Normal.

(i) *Phosphotungstic acid soln.*—20%

(j) *Sulfuric acid.*—ca Normal.

(k) *Zinc sulfate.*—0.05 *N*.

(l) *Ammonium sulfate.*

(m) *Phenol red indicator.*—Rub 100 mg of phenol red in a mortar with 5.7 ml of 0.05 *N* NaOH until dissolved, then add sufficient H_2O to make the volume 100 ml.

Lactic Acid in Milk

PREPARATION OF SAMPLE[2]

(a) *Liquid, whole, or skim milks.*—Weigh 50 g into a 100 ml volumetric flask.

(b) *Dried whole or skim milks.*—Weigh 5 g into a 100 ml beaker and make into a smooth paste with small amount of water. Transfer contents of beaker to a 100 ml volumetric flask with about 50 ml of water.

To the mixtures add 6 ml of normal H_2SO_4 and mix, avoiding vigorous agitation. Add 5 ml of 20% phosphotungstic acid soln and make to mark with water. Shake and filter thru a folded filter paper.

PREPARATION OF SODIUM SALT OF LACTIC ACID[2]

Place 50 ml of filtrate in a continuous extractor, add 0.5 ml of 1+1 H_2SO_4 and extract with ether 3–4 hours. To the contents of the extraction flask add 20 ml of H_2O and evaporate the ether on the steam bath. Add one drop of phenolphthalein soln and make alkaline by dropwise addition of saturated $Ba(OH)_2$ soln. Transfer to a 110 ml volumetric flask, using about 70 ml of alcohol for the transfer. Heat almost to boiling on the steam bath, cool, and make to volume with alcohol. Filter thru a folded filter paper into a 250 ml beaker. Evaporate the filtrate to dryness on the steam bath. Add 20 ml of water and 3 or 4 drops of 1:1 H_2SO_4, stir well, and filter. Wash the beaker and filter with two 5 ml portions of water. Make the filtrate alkaline to phenolphthalein by dropwise addition of 40% NaOH. Evaporate to dryness.

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[1] Mallinckrodt's AR precipitated powder was used in this work.

[2] The preparation of sample, ether extraction, and precipitation of barium salts are described in *Methods of Analysis* (1945) secs. 22.8, 22.12, and 22.13, respectively. They are repeated here for convenience only.

PREPARATION OF THE PARTITION COLUMN

Place 20 g of silicic acid[3] in a mortar, add 5 ml glycerol indicator soln, 3.7 ml of H_2O and N NH_4OH, dropwise, sufficient to produce alkaline color to the indicator (1–3 drops). Mix, using a spatula to break up lumps. With pestle grind into a uniform powder. Make a slurry with 50 ml of 20% butanol-$CHCl_3$ solvent, and transfer with aid of a beaker to a glass tube 25×200 mm, one end of which has been constricted to an outlet 3 cm long and 5–6 mm in diam., plugged at the constriction with glass wool, and clamped in an upright position. Apply air pressure (3–5 lbs.) to the large end, forcing the excess solvent dropwise out of the small end (ca 20 ml). Release pressure as soon as the liquid disappears at the top of the gel. (Otherwise gel will dry and crack, becoming useless.) Add 1 ml of $CHCl_3$ containing 20 mg of acetic acid, and 2 ml of butanol-$CHCl_3$ solvent containing 20 mg of lactic acid. Apply pressure until the surface of the solvent just disappears into the gel. Fill the tube with solvent, place a 100 ml graduated cylinder beneath the outlet and apply pressure. Collect percolate until the lower edge of the blue band, second from the outlet end, reaches the constriction of the tube. The volume collected is the threshold volume (s) for lactic acid. (The first blue band to leave the column is the acetic acid.)

ISOLATION OF LACTIC ACID

To the dry residue of sodium lactate in the 50 ml beaker, add 2 ml of butanol-$CHCl_3$ solvent, 5 or 6 drops H_2SO_4 (1+1), mix well with glass rod, breaking up all lumps, and stir in 1 g of anhydrous Na_2SO_4.

Prepare a new partition column from the same batch of silicic acid, proceeding as directed under "Preparation of the Partition Column" above, down to and including the addition of the 1 ml of $CHCl_3$ containing 20 mg of acetic acid. Place a 100 ml graduated cylinder beneath the outlet of the tube. With a pipet, transfer the solvent containing the lactic acid to the column and apply pressure until the liquid disappears into the gel. Wash the beaker with three successive 1 ml portions of solvent, transferring to the column with the same pipet, and applying pressure each time until the liquid disappears. Fill the tube with solvent and apply pressure. A light placed behind, but not so close as to heat, the tube increases the visibility of the bands because of the translucency of the gel formed by glycerol and silicic acid.

Collect percolate until the bottom edge of the second band reaches the constriction of the tube, and discard. Place a 50 ml graduated cylinder beneath the outlet, and collect percolate until the second blue band has passed out of the column (not less than 35 ml).

With 2 mg of lactic acid or less, the second blue band may not be distinct enough to be visible. In such case remove and discard the percolate equal in volume to the threshold volume (s) for lactic acid established for the silicic acid being used. Place a 50 ml graduated cylinder beneath the outlet and collect 35 ml of percolate.

Transfer the 35 ml of percolate to a 125 ml Erlenmeyer flask with 20 ml of H_2O and titrate with 0.05 N $Ba(OH)_2$ soln (phenol red indicator). One ml 0.05 N alkali =4.5 mg lactic acid.

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[3] The optimum amount of silicic acid, for the quantities of glycerol and water used above, may vary with different lots of silicic acid. *See* Ramsey & Patterson, *This Journal*, p. 142.

IDENTIFICATION OF LACTIC ACID

Separate the aqueous layer in the titration flask. Add 0.05 *N* zinc sulfate equivalent to the $Ba(OH)_2$ used in the titration. Heat to boiling and boil for 5 min., stir in a small amount of Norite and filter. Evaporate to dryness on the steam bath. Add 10 ml of acetone, heat to boiling and decant. Repeat heating and decantation once more with acetone, and then once with alcohol. Add a small volume of water, and filter if the solution is not clear. Evaporate on the steam bath to a concentration of 5 mg of lactic acid per ml. Place a few drops of this solution on a microscope slide, allow the water to evaporate at room temperature and observe the crystals of zinc lactate. For amounts of lactic acid less than 5 mg, leave the solution in the beaker and allow the water to evaporate at room temperature. Place the beaker under the microscope and examine the crystals. Compare the crystals with those from a known solution of pure zinc lactate.

Succinic Acid in Egg Products

PREPARATION OF SAMPLE

(a) *Liquid eggs.*—Transfer 200 g to a tared 1500 ml beaker. With constant stirring, add 500 ml of H_2O, 75 ml of *N* H_2SO_4, and 125 ml of 20% phosphotungstic acid soln. Make contents to 1000 g by addition of H_2O, stir thoroly, and filter thru a folded filter paper.

(b) *Dried eggs.*—Weigh 50 g into a tared 1500 ml beaker and stir into a uniform paste with 100 ml of H_2O. With continuous stirring, add 600 ml of H_2O, 50 ml of *N* H_2SO_4 and 75 ml of 20% phosphotungstic acid soln. Make contents to 1000 g with H_2O, stir thoroly, and filter thru a folded filter paper.

PREPARATION OF SODIUM SALT OF SUCCINIC ACID

Evaporate 500 g of the filtrate in a 1000 ml beaker on a hot plate to ca 75 ml, and concentrate on a steam bath to ca 20 ml. Transfer to the continuous extractor by aid of 10 ml of H_2O. Add 23 g of $(NH_4)_2SO_4$ and 1 ml $H_2SO_4(1+1)$ to the soln in the extractor. Rinse the beaker with ether into the extractor, and extract with ether for 2½ hours.

Add 20 ml of H_2O to the extraction flask and expel the ether on steam bath. Neutralize with saturated $Ba(OH)_2$ soln (phenolphthalein indicator), transfer to 110 ml volumetric flask with alcohol until volume is ca 90 ml. Heat almost to boiling on steam bath, cool, complete volume with alcohol and filter thru folded 12½ cm filter paper. Place the paper and precipitate in a 50 ml beaker, add 20 ml of H_2O, 3 or 4 drops of $H_2SO_4(1+1)$, heat on steam bath ca 5 min., and filter. Wash the beaker and its paper with 3 successive 8 ml portions of H_2O, pour thru the filter, collecting filtrate and washings in a 50 ml beaker. Neutralize the filtrate with 40% NaOH soln (phenolphthalein indicator) and evaporate to dryness on steam bath.

ISOLATION OF SUCCINIC ACID

Prepare a partition column as described for lactic acid, using a soln of 20 mg of succinic acid in 2 ml of butanol-$CHCl_3$ solvent instead of the lactic acid soln to determine the threshold volume for succinic acid. Prepare another column, acidify the sodium succinate, and put the soln thru the partition column, in the same manner as described for "Isolation of Lactic Acid." Transfer the 35 ml percolate to a 150 ml Erlenmeyer flask with 20 ml of H_2O and titrate with 0.05 *N* $Ba(OH)_2$ soln (phenol red indicator). One ml N/20 alkali = 2.95 mg succinic acid.

IDENTIFICATION OF SUCCINIC ACID

Separate the aqueous soln in the titration flask, transfer to a 50 ml beaker,

evaporate to about 3 ml on the steam bath, and then allow to evaporate at room temperature until crystals appear. Compare the crystals with known barium succinate prepared by titrating about 25 mg of pure succinic acid with the $Ba(OH)_2$ soln, adding 3 or 4 drops of glycerol, and evaporating as directed above.

DISCUSSION

Methods for the determination of lactic and succinic acids in which partition chromatography is an essential step, have been developed. When lactic acid was added to samples of good powdered milk, which were shown to contain no lactic acid as measured by this procedure, the recoveries as shown in Table 1, range from 97 to 99 per cent. In order to determine if any lactic acid can be detected in fresh milk by this method, the acids extracted from 250 grams of milk were put on a column. That portion of the percolate which would have contained any lactic acid present gave a titration equivalent to 0.9 mg of lactic acid; but no crystals of the zinc salt could be identified by microscopic examination.

TABLE 1.—*Recovery of lactic acid added to dried whole milk (5 grams)*

LACTIC ACID ADDED	LACTIC ACID FOUND	RECOVERY
mg	*mg*	*Per cent*
6.5	6.5	100
13.0	12.7	97
26.0	25.6	98
39.0	38.6	99
52.0	51.0	98

When succinic acid was added to liquid and dried eggs, the recoveries, as shown in Table 2, were in the range 86 to 95 per cent No succinic acid was found in these eggs when they were analyzed by the same procedure.

When a mixture of α-hydroxybutyric, β-hydroxybutyric, lactic and glycolic acids was placed on this partition column, they separated and came off in the order named. The separation of β-hydroxybutyric and lactic acids was not complete; however, they did separate into two bands which would make a mixture of the two acids easily detectable.

When a mixture of acetic, formic, and lactic acids was placed on the column the acids came off in the order named. They separated into well-

TABLE 2.—*Recovery of succinic acid added to liquid and dried eggs*

PRODUCT	WT. SAMPLE	SUCCINIC ACID ADDED	RECOVERY
	grams	*mg*	*Per cent*
Dried eggs	50	29.5	87, 86
Liquid eggs	200	50.0	88
Liquid eggs	200	2.0	93
Liquid eggs	200	4.0	95

defined bands which makes complete separation possible. If these acids are placed on the column with α-hydroxybutyric and β-hydroxybutyric acids, the α-hydroxybutyric and acetic acids come off together, and the β-hydroxybutyric and formic acids come off together. Citric and malic acids cause no interference, since citric acid remains at the top of the column, and malic moves so slowly that it is hardly possible to wash it off at all. Succinic acid moves down the column at a rate slightly faster than lactic acid, but the difference in the threshold volumes of the two acids is not great enough for complete separation. These two acids may be separated by precipitation of the barium salts in 80 per cent alcohol; the precipitate may then be used for the determination of succinic, and the filtrate for the determination of lactic acid.

BIBLIOGRAPHY

(1) Friedmann, T. E., Cotonio, M., and Shaffer, P. A., *J. Biol. Chem.*, **73**, 335 (1927).

(2) Troy, H. C., and Sharp, P. F., *Cornell Agri. Exp. Sta.*, Memoir 179–228 (1935)

(3) Mendel, B., and Goldscheider, I., *Biochem. Z.*, **164**, 163 (1925).

(4) Barker, S. B., and Summerson, W. H., *J. Biol. Chem.* **138**, 535 (1941).

(5) Hillig, F., *This Journal*, **20**, 130 (1937).

(6) Hillig, F., *Ibid.*, **25**, 253 (1942).

(7) Winton, A. L., and Winton, K. B., "The Analysis of Foods," pp. 219, 674 (1945).

(8) Weil-Malherbe, H., *Biochem. J.*, **31**, 299 (1937).

(9) Krebs, H., *Ibid.*, **31**, 2095 (1937).

(10) Ramsey, L. L., and Patterson, W. I., *This Journal*, **28**, 644 (1945).

(11) Isherwood, F. A., *Biochem. J.*, **40**, 688 (1946).

(12) Liddell, H. F., and Rydon, H. N., *Ibid.*, **38**, 68 (1944).

SEPARATION AND DETERMINATION OF THE STRAIGHT-CHAIN SATURATED FATTY ACIDS C_5 TO C_{10} BY PARTITION CHROMATOGRAPHY*

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The straight-chain saturated fatty acids C_5 to C_{10} can be separated and determined by the classical and time-honored method of distillation: steam distillation plus direct fractional distillation of the readily volatile acids, and then fractional distillation of the methyl or ethyl esters of the less volatile acids. (1) While refinements in apparatus and improvements in procedure have increased somewhat the reliability of this method, fractional distillation often does not produce sharp separations, is time consuming, and requires a rather large sample. Therefore, it is obvious that there is a need for a rapid routine method for the analysis of these fatty acids. There are innumerable potential uses of such a method in research on fermentation, food decomposition, the composition of certain fats, and the composition of mixed esters and flavors, natural or synthetic.

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The method presented in this paper for the separation and determination of the straight-chain fatty acids C_5 to C_{10} is a modification of the chromatographic partition method previously proposed for the lower volatile fatty acids C_1 to C_4 (2, 3). Whereas the fatty acids above butyric are only slightly soluble in water, their solubility in methanol is appreciable, and they are also soluble in aliphatic hydrocarbons such as isooctane. These two solvents were found to be satisfactory for use as the immiscible solvents in separating the fatty acids C_5 to C_{10} by partition chromatography. The silicic acid is almost saturated with methanol containing bromocresol green as the indicator, slurried with isooctane, poured into a suitable tube of the type normally used in chromatography, and allowed to pack down under pressure. Other hydrocarbons such as n-hexane and cyclohexane can be used in place of the isooctane but the bands on the column are less distinct with these solvents. When an isooctane solution containing the straight-chain acids: valeric, caproic, enanthic, caprylic, pelargonic, and capric is passed through this column, a separation into six well defined bands is obtained, analogous to that for the lower volatile fatty acids (2). The valeric acid band is found at the top of the column, the capric acid band at the bottom, and the others in order between these two. If as many as three 5-carbon acids (n-valeric, isovaleric, and α-methylbutyric), and three 6-carbon acids (n-caproic, isocaproic, and β-methylvaleric) are all present in a mixture, each group of acids with the same number of carbon atoms behaves as a single acid, *i.e.*, each group forms a single band on the column. Thus, the method presented here will separate a mixture containing only the straight chain saturated fatty acids C_5 to C_{10}.

After the chromatographic separation of the straight-chain fatty acids C_5 to C_{10}, the individual fatty acid in each band can be tentatively identified by its threshold volume (4). The threshold volume for a given compound is an empirical figure which may be defined as the volume of percolate collected under specified conditions while the compound is moving from the top of the column to the point of egress at the bottom. The threshold volumes for the fatty acids C_5 to C_{10} are quite reproducible, and characteristic of the individual acids. Therefore, it is feasible, using pure acids, to standardize the column with respect to the threshold volumes of the acids. The identification of a fatty acid can be confirmed by admixture with an approximately equal amount of an authentic sample of the suspected acid, followed by passage of this admixture through a column of suitable size prepared in the same way. The formation of only one band then indicates that the isolated acid is identical with the authentic specimen, or at least is very closely related; *i.e.*, it very probably contains the same number of carbon atoms.

No acids except the branched-chain acids C_5 to C_{10}, and possibly the unsaturated acids C_5 to C_{10}, in the fatty acid series are known or expected to interfere in the method. The behavior of the unsaturated fatty acids C_5 to C_{10} on the column, however, was not investigated. Acids such as oxalic, succinic, malic, citric, and tartaric, if placed on the column, remain practically stationary at the top since they are almost insoluble in isooctane. Homologs in the fatty acid series below valeric and above capric do not interfere. Formic, acetic, propionic and n-butyric acids move much more slowly on the column than valeric; and by the time the valeric acid has moved completely through the column these acids will have separated into three bands, a n-butyric band, a propionic band, and a band containing both acetic and formic acid. The separation of the valeric and n-butyric acids appears to be quite complete. Hendecanoic (undecylic), lauric, and myristic acid, when present to the extent of about 10 mg each, will separate into visible bands, but the bands are so close together that the separations are not complete. However, the separation of capric from undecylic appears to be fairly complete, and the separation of capric from lauric quite complete. Acids higher than myristic do not interfere in the method, but when present in great excess as compared with the acids C_5 to C_{10}, it is necessary to collect the C_5 to C_{10} acids as a single group, and then to refractionate on a fresh column. The indicator appears to become quite insensitive to small amounts of acid after a large amount of acid has passed through the column.

How pure the acid in each eluted band may be is difficult to establish on a few milligrams by the usual criteria of purity. It requires about 2 milligrams of acid (about 2 for capric and somewhat less for valeric) to make a visible band on a column 1 inch in diameter and containing 20 grams of gel; for a 2 gram column in a smaller tube only about 0.2 milligram is required. Thus, on the larger column 2 milligrams of residual acid might remain unnoticed after passage of the visible band. When a single acid is passed through the column, the volume of eluate necessary to recover all of the added acid is a little larger than the volume apparently required to elute the same acid from a mixture. Whether, in a mixture, a following band tends to push the one in front of it through faster (in a smaller volume) than the front band would move, if alone, or whether the following band tends to engulf the tail of the one in front of it, is a difficult question to answer. However, when a mixture of the six straight-chain acids C_5 to C_{10} was separated by partition chromatography and each of the separated acids refractionated on a fresh column, each acid was found to contain about 5% of the acid which immediately preceded it on the column. To test more accurately the completeness of separation of any given acid in the C_5 to C_{10} group from its homologs by two or more passages through a partition column, chromatography of a mixture of the acid with a synthetic sample of each homolog containing a radioactive atom as a tracer, could be employed.

The working range of the method presented here is 2 to 20 milligrams of each acid in the solution transferred to the column. When only even numbered carbon acids, or only odd numbered carbon acids, are being separated, the capacity of the column is appreciably greater. A column larger than the one used in this analytical method would, of course, have a correspondingly greater capacity. It would be practical to use a column large enough for isolating and purifying several grams of the fatty acids.

Preliminary experiments indicate that the proposed method will be useful in studies on fermentation, decomposition of foods, the composition of certain fats, and the composition of ester-containing flavors, both natural and synthetic; and that the method will also be useful in checking the purity of fatty acids, and possibly in routine control work in connection with the manufacture of the fatty acids.

METHOD

APPARATUS

(1) *Chromatographic tubes.*

- (a) Large size: 27 mm O.D.×200 mm long prepared from Pyrex tubing, or from Pyrex test tubes by sealing into the bottom of each a piece of glass tubing 6 mm O.D.×25 mm long.
- (b) Small size: 18 mm O.D.×250 mm long prepared from 18 mm O.D. Pyrex tubing.
- (c) Any other suitable container.

(2) Suitable pressure source such as compressed air or a cylinder of nitrogen, and a means of keeping the pressure constant such as a column of mercury or a diaphragm type pressure regulator.

REAGENTS

1. *Silicic acid.*[a]

2. *Methanol.*

3. *Isooctane (2, 2, 4-trimethylpentane*[b] *solvent.*—If not free of acid, wash with alkali, dry, and distil. To ca one l. of isooctane add 20 ml of 90% methanol, shake vigorously, allow to separate, and discard the methanol layer.

4. *Bromocresol green solution.*—Dissolve 200 mg of the dye in 25 ml of methanol.

5. *Phenolphthalein soln.*—Dissolve 1 g of phenolphthalein in 100 ml of absolute alcohol and neutralize with sodium ethylate.

6. *Ammonium hydroxide solution,* ca *N.*

7. *Sodium ethylate, standard 0.02 N solution.*—Prepare 0.02 *N* sodium ethylate using aldehyde-free absolute alcohol (5), by the usual procedure (6).

8. *Straight chain fatty acids.*—Valeric, caproic, enanthic, caprylic, pelargonic, capric, and lauric.

PROCEDURE

(1) *Determination of the optimum ratio of methanol to silicic acid:* To 20 g of the sample of silicic acid in a mortar, add 10 ml of methanol and mix well with a pestle.

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[a] Mallinckrodt's Analytical Reagent grade precipitated powder used in this work.

[b] Pure grade product of Phillips Petroleum Company used in this work.

Continue the addition of methanol stepwise in 2 ml portions, mixing well after each addition, until the silicic acid is gummy and sticky; at this point it will not produce a smooth slurry with isooctane. The optimum amount of methanol to be added to the silicic acid usually lies from 2 to 5 ml below this point and must be determined by actually preparing a slurry with the isooctane, transferring the slurry to a chromatographic tube, and observing whether the gel packs down quite firmly under pressure without cracking or drying out.

(2) *Preparation of the chromatographic partition column:* To 1 ml of the bromocresol green soln, add sufficient methanol to give the optimum amount as determined in (1) above and add enough of the ammonium hydroxide soln (1 to 3 drops is usually sufficient) to give the alkaline color of the indicator. Add the methanol soln to 20 g of silicic acid in a mortar and mix thoroughly with a pestle. If the silicic acid is not alkaline enough to be pale bluish-green in color, add more of the ammonium hydroxide solution dropwise, mixing thoroly after each addition, until this color is obtained. Add ca 60 ml of the isooctane and make a slurry. Place a very small cotton plug firmly in the neck of the constriction at the bottom of the large size chromatographic tube, and clamp the tube in an upright position. Pour the slurry into the tube, preferably by transferring from the mortar to a 100 ml beaker and pouring from the beaker into the tube; connect the top to a suitable pressure source through the gas pressure regulator; and apply 2 to 5 pounds of pressure. During the packing down process, tap the tube from time to time to aid in obtaining a smooth, level surface. Release the pressure when the silicic acid has packed down firmly, but before the column begins to dry out, crack, or separate from the walls of the tube. (If the column begins to dry out before the acids are added, remove the gel from the tube, slurry again with the isooctane, retransfer to the tube, and repack.)

(3) *Testing the silicic acid for its suitability and standardization of the chromatographic column:* To neutralize the slight but unavoidable excess of ammonium hydroxide on the column before use, place 2 ml of isooctane containing ca 10 mg of lauric acid on the column, adding carefully from a pipet so that the top surface of the column is not disturbed. Connect to the pressure source and apply pressure. At the instant all the solution has sunk into the gel, release the pressure, disconnect, and add 1 ml of isooctane. Renew the pressure until this wash solvent has sunk into the gel, and repeat the washing again using 1 ml of the isooctane. Pass ca 15 ml of additional isooctane through the column, stopping when all of the solvent has sunk into the gel. The column is now ready for use. Place a graduated cylinder under the column to collect the forerun, including the lauric acid, and pipet onto the column 2 ml of a standard solution in isooctane, of fatty acids, which contains about 10 mg of each acid. Allow the solution to sink into the gel under pressure, and wash with two 1 ml portions of isooctane as above. Fill up the tube with solvent and adjust the pressure so that the rate of percolation is from 3 to 5 ml per minute (rate not critical). (A separatory funnel or other suitable container may be fitted in the chromatographic tube to serve as a reservoir for the solvent.) Change the receiver when the lower edge of a band reaches the constriction of the tube, disregarding the lauric acid band which is caught in the forerun. In general collect the whole band in one fraction, changing receivers when the following band reaches the constriction, and continue until all of the desired bands have passed. In case there is no following band, change receivers when the visible band has passed through the tube, collecting 5 ml fractions, and titrating each. Continue until the titration agrees with the blank value.

Record the volume of percolate for the forerun and for each band. Transfer each of the fractions to an Erlenmeyer flask, add a few drops of the phenolphthalein soln, and titrate with the standard sodium ethylate. Correct the titration for a blank in each case, using approximately the same quantity of isooctane as was used to elute the acid being titrated.

The first band of the mixture to elute is capric acid; the second, pelargonic; the third, caprylic, etc. The threshold volume for capric acid is the volume of the forerun (includes lauric acid band). The threshold volume for pelargonic acid is the forerun volume plus the volume of solvent required to elute the capric acid. Obtain threshold volumes of the remaining acids in a similar manner. These threshold volumes constitute the standardization of the column for 10 mg of each of the fatty acids under the specific conditions actually employed.

Consider the silicic acid suitable if, upon following the above procedure, the acid bands are clearly visible, well separated as determined by visual observation, and if the recoveries of the added acids are in the range 90–110%.

(4) *Determination and identification:* The preparation of the sample will vary from product to product. The first step in any event, however, will be the separation and concentration of the acids either by steam distillation or solvent extraction, or a combination of the two processes. Neutralize the distilled or extracted acids, concentrate to a small volume, and transfer to a test tube. Remove the water in a current of air at steam bath temperature, add 2 to 4 drops of $H_2SO_4(1+1)$ (enough to turn congo red paper to its acid color) and a few glass beads. Wet the solid salts by rotating the glass beads in the tube, using a long thin-bladed spatula to break up the crust or lumps if necessary. Extract the liberated acids with 2, 1, and 1 ml portions of the isooctane; and transfer each portion to the column with an eyedropper pipet, allowing the solvent to sink into the gel each time before the next portion is added.

Collect the fractions, determine the threshold volume of each acid band, and titrate as in (3) above.* Compare the threshold volume of the unknown acid in each case with approximately the same quantity of authentic acid (see (3) above), and thus make a tentative identification of the unknown acids.

To confirm this tentative identification add ca 5 ml of water to the neutralized isooctane solution of each unknown acid, evaporate the isooctane on the steam bath, transfer to a small test tube and continue evaporation to dryness. However, when the volume of isooctane solution is 50 ml or greater, extract the sodium salt from the solvent by shaking with three small successive portions of water in a separatory funnel and drawing off each portion into a small beaker. Concentrate the aqueous solution to ca 5 ml and transfer to a test tube and evaporate as above. Convert the sodium salt of each acid to a solution of the free acid in isooctane as described above, and pipet an aliquot of ca 1 ml, containing ca 2ml of 0.02 *N* acid (4 to 7 mg of the acid), onto a freshly prepared column. Pipet an approximately equal amount of the authentic acid, in not more than 1 ml, onto the column; gently swirl to mix; and proceed to develop the chromatogram. If only one band is obtained, the tentative identification is confirmed. Report the results in mg of acid found.

Table 1 shows the recoveries of the fatty acids when present in admixture in the range 2 to 20 mg each, 5 different mixtures being analyzed, 2 of them in duplicate. As can be seen, recoveries are generally within ± 5% except mixture "E," where the recoveries on 2 mg quantities are high. No corrections have been made for homologs known to be present as impurities in the acids used, but in no case in this table would the results be appreciably affected by such slight impurities.

Table 1 also shows the threshold volumes obtained in each experiment for

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* Where results of the highest degree of accuracy are required, each of the separated acids should be steam distilled and the distillates titrated with standard aqueous alkali (CO_2 free), using phenolphthalein as the indicator. Results reported in this paper, however, were obtained by omitting the steam distillation step.

EXPERIMENTAL RESULTS AND DISCUSSION

TABLE 1.—*Recoveries of the acids C_5 to C_{10} when present in admixture.*

ACID	ADDED	FOUND	RECOVERY	THRESHOLD VOLUME
Name	*mg*	*mg*	*per cent*	*ml*
		Mixture A		
Capric	2.03	1.96	97	47
Pelargonic	2.04	1.86	91	59
Caprylic	2.03	2.04	101	73
Enanthic	2.02	2.16	107	97
Caproic	2.00	1.88	94	130
Valeric	2.04	1.90	93	174
		Mixture B		
Capric	5.08	5.0	98	44
		5.2	102	43
Pelargonic	5.11	4.7	92	55
		5.2	102	55
Caprylic	5.08	5.1	100	70
		5.1	100	69
Enanthic	5.06	5.1	101	90
		5.1	101	90
Caproic	4.99	4.9	98	123
		5.3	106	122
Valeric	5.11	4.8	94	172
		5.0	98	173
		Mixture C		
Capric	10.17	9.5	93	42
		10.1	99	43
Pelargonic	10.22	10.0	98	53
		10.1	99	53
Caprylic	10.15	10.7	105	66
		10.8	106	66
Enanthic	10.12	9.3	92	88
		9.7	96	88
Caproic	9.98	9.5	95	177
		9.6	96	119
Valeric	10.22	9.7	95	167
		9.9	97	159
		Mixture D		
Capric	20.33	19.3	95	41
Pelargonic	20.43	20.2	99	51
Caprylic	20.30	20.4	100	63
Enanthic	20.23	20.6	102	83
Caproic	19.96	18.7	94	112
Valeric	20.43	20.6	101	152
		Mixture E		
Capric	9.91	9.5	96	47
Pelargonic	2.02	2.1	104	60
Caprylic	10.16	9.8	96	73
Enanthic	2.03	2.4	118	100
Caproic	10.05	9.5	95	127
Valeric	2.02	2.3	114	199

each acid. It will be noted that the threshold volume of an acid is definitely affected by the amount of such acid placed on the column; the larger the amount of acid the smaller the threshold volume, *i.e.*, large amounts of an acid travel faster on the column than small amounts of the same acid. In actual practice, then, the quantity of an acid must be taken into consideration in making the tentative indentification by this means.

The threshold volume of an acid is not appreciably affected by large amounts of the homologs of the acid. In carefully controlled experiments using the same amount of a given acid, the threshold volume varied as much as 10% (± 4 ml for capric acid; ± 10 ml for valeric). The cause of these small variations was not definitely determined. However, it was found that the rate of percolation within rather wide limits, 2 to 6 ml per min., did not affect the threshold volume, and that the quantity of methanol within the range 10 to 20 ml per 20 g of silicic acid (using silicic acid from the same lot each time) was also without effect.

The color of the band formed by a definite quantity of capric acid, say 0.02 millimol, is not as strong as that formed by the same quantity of a lower acid. In fact the strength of the color contrast increases noticeably from capric to valeric acid. The fact that only the acid dissolved in the methanol can affect the color of the indicator, and that the distribution of the lower acids favors the methanol solvent, is probably the explanation of this behavior.

Completeness of Separation on the Column:

Since the acid bands widen and "tail out" as the chromatogram is developed, and since, when all six of the acids are present in the mixture being chromatographed, the bands which first elute are fairly close together, it appears that there is a possibility of contaminating a separated acid with its next higher homolog. However, the probability that a separated acid is also contaminated by its next lower homolog is not as great, since it has been found that the front of a visible band is sharp, permitting accurate fraction cuts to be made; and since, if the acid is pure, no acid elutes in front of this visible band.

An experiment was performed to determine the degree of contamination of acids separated by the proposed method, by their next higher homologs. A mixture consisting of ca 10 mg each of the six fatty acids C_5 to C_{10} was separated on a column. The separated acids, except capric and valeric, were refractionated individually on a fresh column, the percolate being collected in small fractions in the zone where the next higher acid, if present as a contaminant, would be found. It should be noted that even if the contamination of one acid with another in this case were as much as 10 per cent, the impurity would not show as a band, because 1 mg of an acid is not enough to produce a clearly visible band. Therefore, it is necessary to collect eluate fractions ahead of the main band in order to detect the presence of the next higher homolog. Results of this experiment are tabulated below, in Table 2. In this experiment the identity of the homolog impurity was not confirmed by fractionation of an admixture with an authentic sample because the amount of impurity was quite small. However, the reliability of the procedure was tested by adding 1 mg of capric acid to 10 mg of pelargonic acid in one experiment and by adding 1 mg of caprylic acid to 10 mg of enanthic acid in another experiment. The recovery of the homolog impurity in both cases by the above procedure was ca 90 per cent.

TABLE 2.—*Degree of contamination of an acid separated by partition chromatography with its next higher homolog*

NAME OF ACID REFRACTIONATED	AMT. OF ACID	FRACTION COLLECTED	FRACTION NORMALLY CONTAINING NEXT HIGHER HOMOLOG*	HOMOLOG FOUND		CONTAMINANT
				NAME		
	ml 0.02 *N*	*ml*			*ml* 0.02 *N*	*per cent*
Pelargonic	2.90	51– 58	47– 59	Capric	0.15	5
		58– 60			nil	
Caprylic	3.55	63– 72	59– 73	Pelargonic	0.15	4
		72– 73			nil	
Enanthic	3.70	80– 92	73– 97	Caprylic	0.14	4
		92– 96			nil	
Caproic	4.09	105–125	97–130	Enanthic	0.19	5
		125–132			nil	

* From mixture A of Table 1.

Based on the recoveries in Table 1 it also appears that the contamination of one acid by another is generally not over 5 per cent, and may be less. Of course, when only the even numbered carbon acids, or only the odd numbered carbon acids, are separated at one time, the bands are farther apart, and the degree of contamination of one acid by another may be then of the order of 1 per cent. However, as pointed out above, the purity of an eluted fatty acid in the amounts used is extremely difficult to determine.

Table 3 shows the sensitivity of the method in detecting the homologs of a fatty acid added to such acid as impurities. In general, by one or more refractionations 0.5 to 1 per cent of an acid as a homolog impurity can be detected. It should be noted that although capric acid must contain about 20 per cent of pelargonic acid before it can be detected in a single passage through the column, suitable refractionation will detect as little as 1 per cent pelargonic in capric acid. The procedure used here is similar to that described below in detail with regard to the application of the method to some commercial products.

TABLE 3.—*Detection of the homologs of a fatty acid added to the acid as impurities*

ACID	HOMOLOG	MINIMUM AMOUNT DETECTABLE	
		SINGLE FRACTIONATION	REFRACTIONATION
name	*name*	*per cent*	*per cent*
Capric	Caprylic	5	—
	Pelargonic	20	1
	Undecylic	10	—
	Lauric	2	—
Caprylic	Caproic	—	0.5
Enanthic	Caproic	2	—
Caproic	Valeric	—	0.5
Valeric	n-Butyric	2	—
	Caproic	5	—

A number of samples of acids were examined by the method to determine their purity. The results are tabulated in Table 4. A weighed quantity of the fatty acid, in the range 200 to 300 mg, was dissolved in ca 5 ml of the isooctane and transferred to the standard 20 g column, the lauric acid used as a neutralizing agent being completely removed before the column was used. The complete forerun plus a small part of the main band (5 to 10%) was collected as fraction 1. The middle portion of the main band was collected as fraction 2 and discarded. At 5 to 10 ml before the next lower homolog should have started to elute, fraction 3 was collected and collection was continued until no more acid would elute. Fraction 1, containing a small amount of the main band and the higher homologs, if any, was refractionated on a fresh column. When visible bands in addition to the main band appeared, they were collected, titrated, identified tentatively, and the identification confirmed as in the method above. If no visible bands appeared, fractions were collected in the zone where the higher homologs should have appeared, if present; the fractions were titrated and if acid of the order of 1 mg or more were found, the fractions were identified as in the method above. Fractions believed to be

TABLE 4.—*Method applied to some commercial products*

ACID	GRADE	HOMOLOGS FOUND AS IMPURITIES
Capric	Pure	2% Lauric Acid
Pelargonic	Pure	None
Caprylic	Pure	0.5% Caproic Acid
Enanthic	Pure	5% Caproic Acid
Enanthic	Pure	2% Caproic Acid
Caproic	Pure	2% Enanthic and 0.5% Valeric Acid
Caproic	Technical	30% n-Butyric;not examined for Valeric Acid
Valeric	Pure	None

mixtures were fractionated again and then identified. Fraction 3 containing the tailings of the acid in the main band plus its lower homologs, if any, was refractionated on a fresh column and the fractions measured and identified in a manner similar to that for fraction 1.

Silicic Acid:

The most critical requirement of this chromatographic method is a suitable silicic acid. Different lots of silicic acid from the same source vary in their chromatographic properties, and not all lots have been found entirely satisfactory. Silicic acid prepared in the laboratory from different lots of commercial waterglass by a definite procedure (3) also has not been entirely satisfactory. In Table 5 is a comparison of different samples of silicic acid. Samples 1 to 4 are different lots from the same source, number 1 being the batch of silicic acid used in this work unless otherwise noted. Samples 5 and 6 are the laboratory prepared products. It should be noted that silicic acid 2, which held the smallest quantity of methanol, gave the smallest threshold volume and would not separate the C_9 and C_{10} acids; on the other hand, silicic acid 6, which had the largest capacity for methanol, gave the largest threshold volume and caused the bands to move farthest apart.

TABLE 5.—*A comparison of different samples of silicic acid*

20 G SAMPLE OF SILICIC ACID	THRESHOLD VOLUME FOR 10 MG OF CAPRYLIC ACID	NOTES
	ml	
1	70	Silicic acid found to be most suitable.
2	32	Would not separate pelargonic from capric acid. Held only 12 ml of methanol instead of 20 ml on 20 g.
3	72	Usable but the acid bands were not as clear as 1.
4	74	Usable but the acid bands were not as clear as 1.
5	98	Acid bands not as clear as 1 but moved farther apart in development. Had greater capacity for methanol; used 30 ml.
6	116	Similar to 5. Had largest capacity for methanol; used 35 ml.

The laboratory-prepared gels were of a finer mesh, and the percolation rate was slower than that of the commercial gels under the same pressure. The percolation rate for a given gel is affected somewhat by the quantity of methanol added to the gel; the greater the amount added, the greater the percolation rate. However, as previously indicated, it was found that a range of methanol all the way from 10 to 20 ml per 20 g powder, in the case of silicic acid number 1, did not affect the threshold volume of caprylic acid.

In brief then, experiments indicate that, in order to be suitable a silicic acid should give, under the conditions outlined in the method, a threshold volume of 60 to 80 ml for 10 mg of caprylic acid; should show clearly visible acid bands when 2 mg of each acid are used; and should be capable of taking up about 100 per cent of its weight of methanol without drying out of the column at the top or bottom during use. However, it appears that the only way to find out whether a silicic acid is suitable is to try it out using the procedure outlined above.

The requirement of a suitable silicic acid is not peculiar to this particular method. A silicic acid prepared according to one procedure (7) was not suitable for the separation of the organic acids of fruit, because this silicic acid was too highly "adsorptive." (8) The preparation of a rather weakly adsorptive silicic acid suitable for separating the fruit acids has been described. (8) However, attempts to duplicate this preparation in another laboratory were unsuccessful. (9)

SUMMARY

A method is presented, based on partition chromatography, for the separation and determination of the saturated straight-chain fatty acids C_5 to C_{10}.

The fatty acids are separated on a column of silicic acid, using methanol as the immobile solvent, 2, 2, 4-trimethylpentane as the mobile solvent, and bromocresol green as the indicator. The separated acids are titrated with standard sodium ethylate and tentatively identified by their threshold volumes; and the identification in each case is confirmed by adding an approximately equal amount of an authentic sample of the suspected acid and testing the chromatographic homogeneity of the mixture on a fresh column.

It appears that the method may be suitable for routine use in the study of fermentation, food decomposition, the composition of certain fats, and the composition of natural and synthetic flavors and esters.

BIBLIOGRAPHY

(1) HILDITCH, T. P., "The Chemical Constitution of Natural Fats," p. 369, (1940).
(2) RAMSEY, L. L., and PATTERSON, W. I., *This Journal*, **28**, 644 (1945).
(3) ELSDEN, S. R., *Biochem. J.*, **40**, 252 (1946).
(4) WEISS, J., *J. Chem. Soc.*, **1943**, 297.
(5) *Methods of Analysis, A.O.A.C.*, **31.24**, p. 497, sixth ed. (1945).
(6) *Ibid.*, **23.29(b)**, p. 354.
(7) GORDON, A. H., MARTIN, A. J. P., and SYNGE, R. L. M., *Biochem. J.*, **37**, 79 (1943).
(8) ISHERWOOD, F. A., *Ibid.*, **40**, 688 (1946).
(9) TRISTRAM, G. R., *Ibid.*, **40**, 721 (1946).

THE DETERMINATION OF FREE TRYPTOPHANE IN MILK, CREAM, AND BUTTER

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Milk contains abundant amounts of the three major components of food-proteins, fats, and carbohydrates; it also contains growth factors needed by many micro-organisms. Since milk does not contain any natural inhibitor of bacterial growth, microbiological activity begins immediately after milking.

Many different types of micro-organisms are commonly found in raw milk or cream. Some of these organisms produce lactic acid almost exclusively, which results in clean sour milk or cream when these micro-organisms are the predominant types present. This so-called "clean sour" milk or cream is preferred for certain uses. Other micro-organisms elaborate proteolytic enzymes which decompose the milk proteins. Still other micro-organisms produce lipolytic enzymes which attack the milk fat and cause rancidity. The inter-relationship of these types of organisms is dependent upon a number of highly variable factors, but under certain conditions they may develop concurrently in milk and cream. In the uncontrolled microbial decomposition of milk and other foods there is, of course, no sharp boundary where a desirable type of chemical action stops and another, and undesirable, one begins.

The chemical reactions involved in the microbial degradation of proteins to amino acids and the resulting changes in these acids are known in a general way (1, 2). The proteins are hydrolyzed by enzymatic activity into simpler substances: proteoses, peptones, amino acids, etc. Although many characteristics of the more complex products of protein hydrolysis, *e.g.*, peptones and polypeptides, are known, the chemical characteristics of the amino acids are more clearly defined and better known.

Certain amino acids can be chemically measured in much smaller quantities than others; tryptophane, in particular, has been the subject of numerous investigations, especially in the field of nutrition, and methods capable of measuring microgram amounts of tryptophane are available. However most of these workers (3, 4, 5 6, 7) have been concerned with the total quantity of tryptophane present, and very little attention has been given to the isolation of small quantities of free tryptophane from natural products.

Tryptophane has been suspected of being connected with decomposition in foods in earlier investigations on indole (8, 9, 10, 11). These investigators inferred that the indole was formed from tryptophane. It seems reasonable to suppose that, in the decomposition of foods, tryptophane is formed first by hydrolysis of proteins and then converted in part to indole. Thus there is a good chance that tryptophane might be present in much larger amounts than the resultant indole. Even though this amino acid is not a final end product in the process of protein degradation, it is logical to reason that the products of the earlier enzymatic breakdown might be found more frequently and abundantly in decomposed food than an end product produced by fewer species of microorganisms. As with any useful chemical criterion, the stage of decomposition at which it appears, and the rate of its formation, and of its disappearance, are very important. This report describes a method for the isolation and determination of free tryptophane in milk, cream, and butter, and presents experiments showing the effect of time and temperature on the formation of free tryptophane in milk and cream.

METHOD FOR FREE TRYPTOPHANE IN MILK, CREAM, AND BUTTER

REAGENTS

Color Reagent.—Dissolve 0.4 g. p-dimethylaminobenzaldehyde in 5 ml acetic acid and mix with 92 ml phosphoric acid and 3 ml conc. HCl. Purify the commercial reagent according to Organic Syntheses Collective Vol. I, p. 214–7, Gilman & Blatt, J. Wiley and Sons, N. Y. (1941).

Hydrochloric Acid.—C.P.—Conc.

Acetic Acid.—C.P.—Glacial. This may be conveniently dispensed from a large separatory funnel on a ringstand.

Lactic Acid Soln.—2.5% W/V.

Dilute Hydrogen Peroxide Soln.—Dilute 1 ml 3% H_2O_2 to 5 ml with H_2O. Prepare fresh daily. (Add 1 drop of this soln to 20 ml of acetic acid immediately before use).

Sodium Hydroxide Soln.—10% W/V.

Acetone—90%.—Redistill before dilution, discarding approximately 5% at the end of the distillation. Neutralize to phenolphthalein with 0.1 *N* NaOH just before using. Dilute 90 ml acetone with 10 ml water.

Alumina Cream.—*Methods of Analysis, A.O.A.C.*, 6th Ed., **34.19(b)**, p. 562. The washing operation may be speeded up by centrifuging. This reagent should have a smooth gelatinous appearance. Solns having the appearance of a suspended powder do not filter satisfactorily.

Standard Tryptophane Soln.—Accurately weigh 20 mg tryptophane and dissolve in ca 100 ml water. Dilute to exactly 200 ml. Dispense from a 10 ml microburette. This solution may be diluted with water to a more convenient working strength. Keep stock soln under refrigeration, and do not use after 3 days. (Tryptophane is difficult to wet and therefore dissolves only slowly. Frequent shaking will hasten solution.)

PREPARATION OF SAMPLE

Cream and Milk.—Weigh 25 g of well mixed sample into a 250 ml centrifuge bottle. If the cream is plastic or very thick, dilute with 5 ml hot water or heat on steam bath until the cream is free flowing.

Butter.—Weigh 50 g of well mixed butter into a 250 ml centrifuge bottle and melt at a temperature not above 100°C. Centrifuge at ca 2000 r.p.m. for several minutes and remove and discard the fat layer with a cream pipette. (Or dissolve the 50 g portion in 50 ml ethyl ether by vigorous shaking, add 50 ml pet. ether, shake, and centrifuge at ca 2000 r.p.m. for several minutes. Remove ether-fat layer by means of a siphon or pipette.) Care must be exercised in order that none of the aqueous phase is removed with the fat.

EXTRACTION AND DETERMINATION OF TRYPTOPHANE

Using 3–4 drops of 1% alcoholic phenolphthalein as an indicator, neutralize the sample by the dropwise addition of 10% NaOH soln. Mix the solution after the addition of each drop of alkali. Discharge the pink color by the dropwise addition of 2.5% lactic acid soln and add 1.0 ml in excess. If the sample is warm, cool to room temperature. Add 100 ml of 90% acetone, shake vigorously for at least two minutes, and centrifuge for several minutes at ca 2000 r.p.m. Carefully inspect the sample after centrifuging; if the precipitated protein layer appears to be fluid, or if there is a liquid layer (except fat) in the bottom of the bottle, add 10 ml water, shake vigorously for two min. and centrifuge again. This treatment must be repeated yet again if the precipitate is still fluid. (Tryptophane may be lost at this point if the acetone extract is decanted from a fluid or liquid layer.) Filter, thru a rapid folded filter paper which will retain the fat, into a 250 ml beaker. (Several ml of butter fat usually separates during the centrifuging and is left on the filter paper and discarded.) Evaporate the filtrate to a volume of 5–7 ml on a hot plate, after addition of glass beads to prevent bumping. If the soln develops a basic reaction during evaporation of the acetone, neutralize and add lactic acid in excess as before. If solns have a cloudy appearance after evaporation to 5–7 ml, repeat the neutralization and heat for several minutes. Allow to cool to room temperature, add 2 ml alumina cream, and filter into a 25 ml glass stoppered graduate (C.S.S. 589 White Ribbon filter paper is satisfactory.) Wash the beaker with 2–3 ml water and pour thru the filter. Continue the washing until the volume of the filtrate is 10 ml. (Alternately, the soln may be filtered thru a cotton pledget, to remove fat, into a graduate containing 2 ml alumina cream, made to 10 ml. vol. by washing, and then filtered or centrifuged. Less time is required by this method.) Shake well, pipet 2.0 ml of the sample soln into a 50 ml beaker, and evaporate to ½ ml or less on a hot plate. (Caution: Do not take to dryness.) Allow to cool to room temperature. Add 1 ml hydrochloric acid and 1 ml of the color reagent, mix by swirling, and allow to stand for 15 ± 1 min. Pour the mixture into a dry, 25 ml glass stoppered graduate and wash the beaker with ca 20 ml acetic acid to which has been added 1 drop of the diluted H_2O_2. Make to 25 ml with acetic acid and mix well by inverting the graduate several times. Allow to stand 10 ± 1 min., pour into a suitable photometer cell, and measure the color at 560 mμ.

(While NaCl is often precipitated upon the addition of the strong acids, it generally settles out, leaving a clear supernatant liquid; however, the color solution may be filtered during the final standing if the NaCl does not appear to be settling out.)

(Cream and milk solutions which contain large amounts of tryptophane may require longer than 10 minutes for color development. This condition is easily recognized since a constant reading cannot be obtained. In such instances repeat the determination, using a smaller aliquot.)

In each series of determinations on milk, cream, or butter, determine the blank on an aliquot of each different sample soln in the same manner, omitting only the addition of the color reagent.

Since the fat contents of milk, cream, and butter are so different, calculate all results to the fat-free basis for ease of comparison.

The following formula is convenient for calculating the results:

$$\text{Tryptophane—fat-free basis p.p.m.} = \frac{A \times (B/C)}{g.(100 - F)}$$

Where A = net micrograms tryptophane in aliquot
B = total ml of sample solution
C = ml of aliquot used
G = grams of sample used
F = per cent fat in sample

DISCUSSION OF METHOD

The tryptophane combined in the casein molecule will react with the color reagent and form blue or purplish blue solutions. It is therefore necessary to isolate the free tryptophane resulting from enzymatic or bacterial action in such a manner as will not liberate tryptophane from the casein molecule.

Acetone was selected both as a combined solvent for free tryptophane and a denaturant for interfering milk proteins, after testing a number of common denaturants, *e. g.*, trichloracetic acid, sodium tungstate, sodium chloride, alcohol, etc. The acetone is readily removed by evaporation. One 100 ml portion of the acetone solution is sufficient to extract the free tryptophane completely enough to be within the experimental error of the method.

The varying protein content of milks and creams (and to a lesser extent, of butters), as well as the varying salt content of butters, makes it practically impossible to prescribe an acetone-water ratio which will perform equally well on all samples of milk, cream, and butter. An acetone solution which contains too much water will not satisfactorily coagulate the protein; if, on the other hand, the acetone concentration is too high, the salt solution from the butter will not be dissolved, and significant quantities of tryptophane might then be lost at this step in the procedure.

Unsuccessful attempts were made to dissolve tryptophane in butter fat; further, no tryptophane was detectable in butter fat from cream which contained large amounts of free tryptophane.

Tryptophane is destroyed by heating in acid solution. This effect is more pronounced when the strong mineral acids are used, instead of lactic acid, to neutralize the samples. However, the acetone extraction of the prepared samples must be made from acid solutions, since cloudy concentrates which are almost impossible to filter are obtained if the extraction is made from basic solutions. The acidity recommended approximates the titratable acidity of fresh cream.

A number of reactions have been proposed for the determination of tryptophane in protein hydrolyzates, but one of the most widely used determinations, as well as one of the most sensitive, measures the blue color formed when tryptophane reacts with p-dimethylaminobenzaldehyde in the presence of strong acids. A trace of an oxidizing agent. *e.g.*, H_2O_2 or $NaNO_2$, speeds up the reaction. The blue color can be measured photometrically at 560 mμ.

Sample solutions obtained from cream and milk have a yellow color, probably due to the presence of the water soluble milk pigment, riboflavin. Sample solutions from butter have only a tinge of this yellow color, but such solutions exhibit a fluorescence in ultraviolet light which is typical of riboflavin. The blank due to this yellow color in cream or milk sample solutions, in the absence of the color reagent, usually amounts to not more than 1–2 mm, using the 100 mm cell and the neutral wedge photometer (12). A 2 ml aliquot of butter sample solution has no detectable blank. In view of the small magnitude of the error thus introduced into the determination, only two (unsuccessful) attempts were made to eliminate this color.

The selection of a 2.0 ml aliquot of the sample solution in the actual determination has been governed by a number of considerations: (a) The interference due to the natural pigment is slightly more pronounced, and brownish off-colors are often obtained, when aliquots larger than 2.0 ml are used. (b) In sample solutions obtained from salted butter, the salt precipitates upon evaporation of the aliquot and is not redissolved by the strong acids used in developing the color. This interference is not serious in aliquots up to 2.0 ml; however, if larger aliquots are used, the salt must be removed from the color solution by filtration or some other means. (c) The 10 ml sample solution contains a total of 25 mg lactic acid, and when larger (3–5 ml) aliquots are used, the acid must be neutralized before evaporation.

The term "free tryptophane" as used in this paper, means tryptophane reacting substances in the sample solutions. Preliminary experiments with the method of Horn and Jones (6) indicate that the "free tryptophane" determined in milk or cream by this method is actually tryptophane. Although primarily of academic interest, further study of the point is being undertaken.

The method used was systematically investigated with respect to time of reaction, concentration and character of the acid-aldehyde reagent, diluent, presence of water and concentration of the hydrogen peroxide. The following general conclusions are drawn from theses experiments:

1. Fifteen minutes is a generous allotment of time for the initial reaction. After addition of the acetic acid-peroxide, however, the time limits should be observed closely since the color slowly fades.

2. The phosphoric acid-aldehyde color reagent has a slightly greater sensitivity than a solution of the aldehyde in hydrochloric acid.

3. An oxidizing agent has been found necessary to develop the maximum color. The optimum concentration of the peroxide solution is a 1+4 dilution of 3% U.S.P. hydrogen peroxide. Stronger solutions promote rapid fading. Solutions of KNO_2 and KNO_3 are satisfactory for the development of the color. The color developed by these oxidants is more stable than that developed by H_2O_2 but may require 15–60 minutes for maximum development of color.

4. Acetic acid was selected as the diluent, since no color is developed when the solutions are diluted to the necessary volume with water. Probably the other diluents which have been used in this reaction would be equally satisfactory.

5. The final sample solution is concentrated in order to remove the excess water, which causes fading and, when more than 1 ml is present, results in the development of less color. This fading is not diminished by the use of larger amounts of color reagent and hydrochloric acid.

6. The concentration of p-dimethylaminobenzaldehyde in the color reagent is not particularly critical.

7. The quantities of hydrochloric acid and color reagent used in developing the color are not critical. The amounts indicated have been found to be adequate for as much as 300 mmg tryptophane.

8. It is preferable to finish the determinations on the same day that they are begun; however, the solutions may be stored overnight under refrigeration.

9. When the quantity of tryptophane measured is greater than 10 mmg, a straight line is obtained by plotting photometer readings against micrograms tryptophane.

TABLE 1.—*Recovery of tryptophane added to cream and butter*

SAMPLE	TRYPTOPHANE P.P.M.			
	Present	*Added*[1]	*Total*	*Recovery*
Cream	0	1.0	1.0	1.1
Cream	0	2.0	2.0	2.4
Cream	0	3.0	3.0	3.0
Cream	0	4.0	4.0	4.1
Cream	0	4.0	4.0	4.1
Cream	0	6.0	6.0	5.6
Cream	0	20.0	20.0	17.6
Cream	0	1.0	1.0	1.0
Butter	1.0	1.0	2.0	1.8
Butter	1.0	1.0	2.0	1.9
Butter	1.5	1.0	2.5	2.2
Butter	1.1	1.5	2.6	1.3
Butter	1.3	3.0	4.3	3.8
Butter	1.4	1.0	2.4	2.2

[1] Multiply by 25 to obtain micrograms of tryptophane added to cream sample, and by 50 for butter sample.

RECOVERY EXPERIMENTS

Table 1 shows the recovery of tryptophane added to cream and butter, using the above procedure. The data indicate that the method is sufficiently accurate to detect small differences in the tryptophane content of cream and butter.

EXPERIMENTAL

The following experiments were undertaken to show that free tryptophane actually is formed in the microbial degradation of milk proteins:

Two lots of milk of known history were used in obtaining the data presented in Table 2, and in Fig. 1.

Milk and cream samples A, B, and C were all from one lot of milk sampled approximately four hours after milking at a receiving plant in Crosby, Mississippi. Cream samples B and C contained 38 per cent and

56 per cent fat, respectively. Both were separated from the same milk represented by sample A.

Sub-samples of the milk and of the two cream samples, consisting of 3 sets of 12 half-pint portions and 3 sets of half gallon portions, were placed in sterile containers. One set of each sub-sample was stored at 37°C (98.6°F); the second was held at room temperature which ranged from 18–25°C (64.5–77°F); and the third was placed in a refrigerator at a temperature of 0–4°C (32–39°F). Tryptophane and titratable acidity (13) determinations were made at the time intervals indicated in Table 2. The one-half gallon samples were stirred thoroughly before each analysis. The one-half pint samples were well mixed immediately before analysis, and a different bottle was used for each set of tryptophane and acidity determinations. Thus, the one-half pint samples were aged without intermittent mixing, and the one-half gallon samples were mixed prior to each determination.

Efforts were made to obtain a homogenous mixture throughout these experiments. However, the cream layer on the milk was not completely miscible when the acidity reached ca 0.3 per cent. No difficulty was encountered in obtaining visual homogeneity in the cream samples until the acidity reached 0.4 per cent. Separation was noted both in the milk and in the 38 per cent fat cream samples after standing undisturbed for 12 hours; and it was increasingly difficult to reincorporate the upper layer as the acidity increased. It is possible that some of the inconsistences in the results may be attributed to the difficulty of obtaining perfect homogeneity.

Milk sample D was from a milking observed by the writer at a New Orleans, Louisiana, dairy. Cream sample E was from the same dairy, separated 12 hours earlier and placed in the cooler immediately after separation. Triplicate sub-samples of D and E were placed in sterile one-half pint jars; one set of each was aged as before at 0–4°C; another at room temperature, 23–29°C. (73–84°F); and the third at 37°C.

Inspection of these tables shows that the tryptophane content, and, of course, the titrable acidity of both milk and cream increases with age. The rate of formation of tryptophane and acid increases with rise in temperature. Only the cream data have been used in preparing Fig. I, because it was possible to follow the cream decomposition for longer periods than the milk. This graph clearly illustrates the effect of time and temperature upon the formation of tryptophane in cream. The data given in Table 2 show that milk behaves in the same manner.

From a comparison of the tryptophane content of milk sample A, and cream samples B and C held at 37°C. for periods up to and including 36 hours, it can be seen that the formation of tryptophane in independent of the size of the sample, and is not significantly influenced by the fat content of the sample.

TABLE 2.—*Effect of time and temperature on development of tryptophane and titratable acidity*

AGE (HRS.)	0–4° C.		18–25° C.		37° C.	
	TITRATABLE ACIDITY (% LACTIC)	FREE TRYPTOPHANE P.P.M.	TITRATABLE ACIDITY (% LACTIC)	FREE TRYPTOPHANE P.P.M.	TITRATABLE ACIDITY (% LACTIC)	FREE TRYPTOPHANE P.P.M.
SAMPLE A, MILK—5% FAT						
4	(See text)...	....	0.17	0.9......	....	....
12	0.19 (0.18)	2.1 (1.0)[1]	0.19 (0.21)	1.8 (1.6)	0.23 (0.22)	1.7 (1.7)
24	0.17 (0.18)	1.2 (1.8)	0.50 (0.36)	3.6 (1.8)	0.72 (0.77)	5.3 (4.8)
36	0.17 (0.17)	0.5 (1.1)	0.80 (0.74)	3.1 (2.7)	0.88	6.3
48	0.18 (0.16)	1.0 (1.2)	0.85	4.2	0.91	7.8
96	0.14 (0.17)	1.9 (1.4)				
144	0.17 (0.17)	1.2 (1.2)				
192	0.16 (0.17)	1.7 (1.3)				
240	0.18 (0.17)	1.6 (1.3)				
288	0.18 (0.19)	2.8 (2.0)				
336	0.20 (0.18)	5.8 (3.0)				
408	0.32 (0.22)	8.4 (3.9)				
480	0.42 (0.38)	7.8 (9.3)				
SAMPLE B, CREAM—38% FAT						
4	(See text)...	....	0.08	0........	....	....
12	0.11 (0.13)	1.2 (0)	0.12 (0.13)	1.0 (1.0)	0.15 (0.16)	1.5 (1.1)
24	0.12 (0.13)	0.8 (0)	0.22 (0.33)	1.9 (1.3)	0.55 (0.56)	3.1 (3.5)
36	0.11 (0.12)	0 (0)	0.54 (0.56)	1.0 (2.8)	0.61 (0.61)	6.3 (5.8)
48	0.12 (0.13)	0 (1.1)	0.60 (0.61)	2.4 (3.1)	0.63 (0.63)	7.7 (7.3)
96	0.13 (0.13)	1.0 (1.0)	0.48 (0.67)	5.8 (5.9)	0.81 (1.02)	16.2 (18.4)
144	0.13 (0.13)	1.6 (1.0)	0.74 (0.73)	11.0 (9.4)	1.08 (1.31)	32.3 (34.8)
192	0.12 (0.12)	1.3 (1.0)	0.80 (0.81)	15.9 (11.6)	1.09	55.9
240	0.13 (0.12)	1.1 (1.6)	0.81	19.2	1.29	82.6
288	0.13 (0.11)	1.4 (1.1)	0.88	23.6	1.42	139.4
336	0.15 (0.13)	1.0 (1.6)	0.95	27.0	1.42	135.5
408	0.29 (0.15)	5.5 (1.6)				
480	0.51 (0.25)	8.5 (2.4)				
SAMPLE C, CREAM—56% FAT						
4	(See text)...	....	0.08	0........	....	....
12	0.07 (0.10)	1.0 (0)	0.09 (0.11)	0 (0)	0.11 (0.10)	0 (1.4)
24	0.09 (0.09)	1.0 (0)	0.20 (0.25)	1.7 (1.1)	0.38 (0.43)	4.7 (3.7)
36	0.09 (0.09)	0 (0)	0.35 (0.37)	1.7 (2.2)	0.40 (0.33)	6.9 (7.0)
48	0.08 (0.10)	0 (0)	0.41 (0.40)	3.0 (4.1)	0.42 (0.40)	11.9 (10.1)
96	0.11 (0.10)	1.0 (1.4)	0.46 (0.41)	6.8 (6.2)	0.64 (0.52)	35.5 (21.7)
144	0.11 (0.10)	1.1 (0)	0.50 (0.48)	12.0 (9.6)	0.92 (0.62)	56.8 (30.0)
192	0.09 (0.09)	1.1 (0.8)	0.50	13.8	0.97	74.6
240	0.09 (0.10)	1.5 (1.4)	0.64	18.3	1.09	102.7
288	0.09 (0.10)	1.5 (1.2)	0.72	26.4	1.15	145.5
336	0.15 (0.15)	2.6 (1.4)	0.83	31.9	1.63	136.4
408	0.22 (0.23)	4.0 (2.6)				
480	0.26 (0.39)	8.5 (3.7)				
SAMPLE D,[2] MILK—3.5% FAT						
0	(See text)...	....	0.15	1.2......	....	....
6	0.17	1.1	0.17	1.3	0.18	1.7
12	0.17	1.1	0.17	0.9	0.17	1.5
18	0.17	1.0	0.16	1.3	0.21	3.2
24	0.16	0.9	0.17	2.2	0.36	3.9
30	0.17	0.9	0.19	3.2	0.59	4.5
36	0.17	0.9	0.21	4.2	0.77	7.8
48	0.16	1.9	0.75	6.8	0.81	11.6
72	0.17	1.4	0.91	7.0	0.85	12.2
96	0.16	1.3				
120	0.18	1.2				
168	0.17	1.4				
SAMPLE E,[2] CREAM—21.3% FAT						
0	(See text)...	....	0.13	0.9......	....	....
6	0.16	0.9	0.16	0.8	0.16	1.1
12	0.16	0.6	0.16	0.9	0.21	1.7
18	0.16	0.8	0.19	1.1	0.64	4.8
24	0.16	0.8	0.55	2.4	0.70	8.0
30	0.16	0.8	0.69	3.9	0.74	8.9
36	0.16	0.8	0.70	4.0	0.74	11.7
48	0.15	1.2	0.76	6.3	0.77	14.4
72	0.17	0.8	0.79	8.0	0.79	21.9
96	0.15	1.2				
120	0.17	1.2				
168	0.15	0.9				

NOTE: Tryptophane values calculated to fat-free basis.

[1] Values enclosed in parentheses were obtained from ½-gallon sub-samples.

[2] 23–29°C. (Room temperature in these experiments.)

The samples held under refrigeration remained fresh; the tryptophane content and titratable acidity remained reasonably constant up to 240 hours. Thereafter a slow increase in both tryptophane and acidity is noticeable. There are greater variations between the ½ gallon and the ½ pint subsamples after 336 hours of storage and, with the exception of sample A at 480 hrs., higher tryptophane values were obtained on the ½ pint jars which had been held without mixing. In some cases there was also a noticeable difference in the acidity. No logical explanation, except the erratic growth habits of micro-organisms at low temperatures, is offered for this behavior.

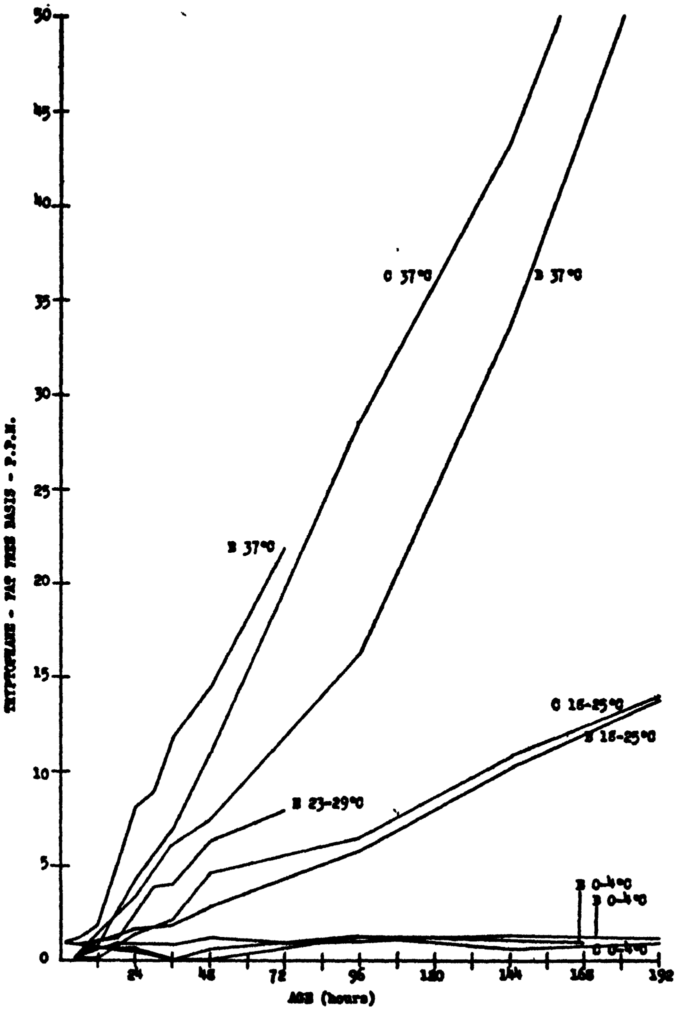

Fig. 1—Effect of temperature on rate of development of tryptophane in cream.

The samples held at room temperature also show an increase in tryptophane content with age. The rate of formation of tryptophane and titratable acidity is noticeably greater than in the samples held at refrigerator termperatures. However, the milk was clabbered at the end of 36 hours, thus making sampling difficult at an early stage of this experiment. The ½ gallon of 56 per cent cream was granular in appearance, and therefore difficult to sample at the end of 48 hours storage. However, measurable quantities of tryptophane were found in the milk before the clabbering point was reached.

The rate of formation of tryptophane and acid was noticeably hastened by incubation at 37°C. The milk samples were clabbered at the end of 24 hours, and, judging from the acidity, had been clabbered for some time before.

TABLE 3.—*Free tryptophane content of butter made from cream of known age*

SAMPLE	AGE (DAYS)	CREAM		BUTTER
		TITRATABLE ACIDITY (% LACTIC)	FREE TRYPTOPHANE FAT-FREE BASIS P.P.M.	FREE TRYPTOPHANE FAT-FREE BASIS P.P.M.
I	0	0.09	0	0
	2	0.48	7.5	7.5
	4	0.57	9.9	9.8
	7	0.76	19.6	13.1
	10	0.95	28.0	23.8
	46	1.34	123.3	—
II	0	0.09	0	0
	3	0.90	10.3	9.2
	6	1.49	28.7	23.8
	14	1.86	77.5	56.0

Table 3 shows the results of similar experiments where cream was stored at room temperature and churned by hand into butter at intervals up to 46 days. These data show that there is a rather close relationship between the free tryptophane content of butter and that of the cream from which the butter was made.

TRYPTOPHANE CONTENT OF COMMERCIAL MILK AND CREAM

In order to gain some information on the normal content of free tryptophane in sweet milk and cream, 18 samples of milk and 22 samples of cream were analyzed. These samples were collected from retail stores in New Orleans, Louisiana, and represent the output of as many of the dairies in this area as could be readily sampled. Each sample represents either a different dairy or a different day's output. These results are presented in Table 4. The three samples of sour cream are representative of the product prepared by inoculating pasteurized sweet cream with a starter. Re-

TABLE 4.—*Free tryptophane content of market milk and cream*

PRODUCT	SAMPLE NO.	TITRATABLE ACIDITY, % LACTIC	FREE TRYPTOPHANE FAT-FREE BASIS P.P.M.
Milk	1	0.11	0.6
	2	0.12	1.0
	3	0.12	0.8
	4	0.10	1.0
	5	0.10	0
	6	0.11	1.0
	7	0.12	0.8
	8	0.10	0.9
	9	0.12	0.7
	10	0.08	1.2
	11	0.10	1.5
	12	0.10	0.9
	13	0.10	1.7
	14	0.10	1.0
	15	0.10	1.0
	16	0.11	1.6
	17	0.13	1.0
	18	0.14	0.9
Breakfast Cream	1	0.11	0.7
	2	0.08	0.8
	3	0.10	2.7
	4	0.09	0
	5	0.10	0
	6	0.10	0
	7	0.10	0.8
	8	0.10	0.8
	9	0.11	2.0
	10	0.12	0.7
	11	0.06	1.5
	12	0.12	2.1
	13	0.09	0.8
	14	0.21	2.4
	15	0.16	1.2
	16	0.09	2.0
	17	0.06	0.7
Whipping Cream	18	0.14	0
	19	0.10	1.0
Sour Cream	20	0.94	4.4
	21	1.05	2.2
	22	0.70	1.3

turned milk and cream are used in the preparation of this product. The free tryptophane in these samples is negligible, compared to the values obtained on raw milk or cream allowed to reach corresponding acidities.

SUMMARY AND CONCLUSIONS

An investigation has been made of the occurrence of free tryptophane in milk, cream, and butter. A method for the extraction and measurement of the free tryptophane in these products has been described.

The following conclusions have been reached:

(1) Negligible quantities of free tryptophane are present in normal sweet milk and cream.

(2) The amount of free tryptophane in milk and cream increases with age if the products are held under conditions conducive to bacterial and enzymatic activity.

(3) The amount of free tryptophane in butter depends upon the free tryptophane content of the original cream.

ACKNOWLEDGMENTS

The author is indebted to J. O. Clarke, Chief, Central District, for his invaluable suggestions throughout the investigation. He also wishes to express his appreciation for the indispensable aid of M. E. Warren and W. A. Bell of New Orleans Station.

REFERENCES

(1) PORTER, J. R., "Bacterial Chemistry & Physiology," pp. 849–889, J. Wiley & Sons, New York (1946).
(2) HARROW and SHERWIN, "Test book of Biochemistry," p. 343, W. B. Saunders Co., Philadelphia (1935).
(3) BATES, R. W., Proc. Am. Soc. Biol. Chem., *J. Biol. Chem.*, **119**, p. vii (1937).
(4) SULLIVAN, M., MILENE, H. S., and EVERITT, E. L., *Ibid.*, **125**, 471 (1937).
(5) SHAW, J. L. D., and MCFARLANE, W. D., *Ibid.*, **132**, 387 (1940).
(6) HORN, M. J., and JONES, D. B., *Ibid.*, **157**, 153 (1945).
(7) GRAHAM, C. E., *et al.*, *Ibid.*, **168**, 711 (1945).
(8) CLARKE, J. O., *et al.*, *This Journal*, **20**, 475 (1937).
(9) KING, W. H., FLYNN, F. F., GOWANLOCH, J. N., *Ibid.*, **28**, 385 (1945).
(10) BEACHAM, L. M., *Ibid.*, **29**, 89 (1946).
(11) DUGGAN, R. E., and STRASBURGER, L. W., *Ibid.*, **29**, 177 (1946).
(12) CLIFFORD, P. A., and BRICE, B. A., *Ind. Eng. Chem.* (*Anal. Ed.*), **12**, 218 (1940).
(13) *Methods of Analysis, A.O.A.C.*, 6th Ed., **22.4**, p. 303 (1945).

NOTES

Note on the Microscopic Identification of Caffeine

By George L. Keenan*

The microchemical identification of caffeine with mercuric chloride reagent already has been described (1) and the crystalline precipitate has been photomicrographed by Stephenson (2). The precipitate appears as needles, as also does the crystalline precipitate formed with gold chloride reagent.

The purpose of this note is to call attention to a much more distinctive test suggested by Arreguine (3) but considerably simplified as to technic. The method of procedure is as follows: A drop of Lugol's Reagent (iodine-in-potassium iodide) is added to a small amount of the test material on an object slide and stirred in. Then a few fragments of ammonium sulfate are dropped into the preparation. As the drop evaporates at the periphery, numerous dark red to brown to almost black (depending on their thickness) quadrilateral plates will appear, many of these so oriented as to show a box-like habit (Figure 1). They resemble rhombohedra in form and exhibit strong birefringence with crossed nicols. This microchemical test is more characteristic and striking than that obtained with either mercuric chloride or gold chloride reagents.

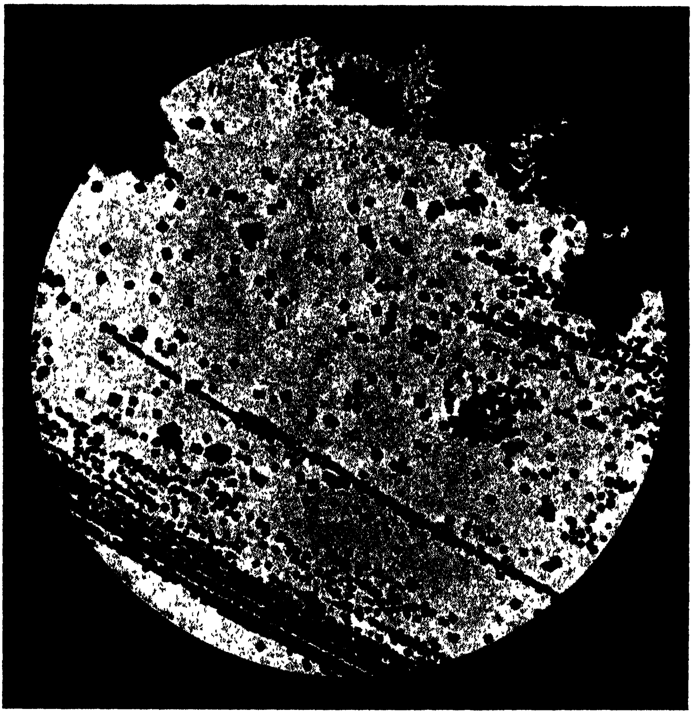

Fig. 1.—Caffeine with Lugol's reagent and ammonium sulfate.

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REFERENCES CITED

(1) *This Journal*, **13**: 46 (1930).

(2) STEPHENSON, CHARLES H.: "Some Microchemical Tests for Alkaloids," J. B. Lippincott Co., 1921, pp. 30–31 (Plate VIII, Fig. 1.)

(3) ARREGUINE, V., *Rev. Univ. nacl.* (Cordoba, Arg.) **29**, 1732 (1942); *Chem. Abs.*, **37**: 6819, Nov. 20, 1943.

Separation of n-Butyric and Isobutyric Acids by Partition Chromatography

L. L. RAMSEY (Food Division,* Food and Drug Administration,
Federal Security Agency, Washington, D. C.)

In applying the method of partition chromatography for separating the straight-chain saturated fatty acids C_5–C_{10} (1) to an unknown mixture of acids derived from a commercial flavoring product, an extraneous acid band was observed between n-butyric acid and n-valeric acid. This extraneous acid was identified as isobutyric acid by the preparation of the characteristic crystalline mercurous salt (2). Previously it was found that a mixture of n-butyric and isobutyric acids does not separate on a chromatographic partition column of silicic acid, using water as the immobile solvent and chloroform containing a small percentage of n-butanol as the mobile solvent (2).

The table below shows the recovery of n-butyric and isobutyric acids when present in admixture, using the same procedure as described for the separation of the straight-chain saturated fatty acids C_5–C_{10} (1). The threshold volumes are also shown. As can readily be seen from the table, the separation of the two acids is not complete. On the column the bands are very close together, in fact, visibly overlapping. A single passage through the column will detect a minimum of about 10% isobutyric acid added to n-butyric acid as an impurity, and about 30% n-butyric acid added to isobutyric acid. Refractionation of appropriate fractions, however, as outlined in the paper on the separation of the acids C_5–C_{10} (1), improves the sensitivity of the method.

The method presented here may be useful in cases where only a small amount of acid is available, where it is desirable to recover the acid after identification, and/or where an approximate determination is satisfactory.

TABLE 1.—*Recovery of n-butyric and isobutyric acids when present in admixture*

NAME OF ACID	ADDED	FOUND	THRESHOLD VOLUME
	mg	*mg*	*ml*
Mixture A			
Isobutyric	10.3	8.2	222
Butyric	10.0	12.3	254
Mixture B			
Isobutyric	5.15	3.7	219
Butyric	5.0	6.4	250

REFERENCES

(1) RAMSEY, L. L. and PATTERSON, W. I., p. 139, *This Journal.*

(2) *Ibid.*, **28**, 644 (1945).

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* W. B. White, Chief.

BOOK REVIEWS

Commercial Fertilizers, Their Sources and Use. By Gilbeart H. Collings. Published by the Blakiston Company, Philadelphia-Toronto, 1947. Fourth Edition, 6⅛×9¼ inches, 522 pages, 160 illustrations, 139 tables. Price $4.50.

The author, Professor of Soils, Clemson Agricultural College, Clemson, South Carolina, and Fertilizer Consultant, Collings and Associates, has had long experience as experiment station agronomist and teacher of soil, fertilizer, and crop courses. Written to satisfy a definite need in the classroom, this book was first published in 1934 with succeeding revised editions appearing in 1938 and 1941. The present new edition, containing eighteen chapters, has been revised to include reference to many changes which occurred in the sources and use of fertilizer materials during World War II. There is an entire new chapter entitled "The Manufacture and Use of Ammonium Nitrate." There are five other chapters on nitrogen, three on phosphate, one on potash, three on other elements in fertilizers including the secondary and minor elements; one chapter each on history, soil reactions and crop growth, purchase of fertilizers, principles of the use of fertilizers; and the final chapter discusses the application of fertilizers and their influence on germination and seedling growth.

In the preface the author states, "Because production and consumption figures for recent years for some countries and states have not been published or are not yet reliable, and because I felt that many conditions as they existed during the war years were not normal, I have purposely used as illustrative material in many chapters the statistics, situations, or practices that prevailed in the normal years just prior to the outbreak of World War II." This treatment of the subject matter is desirable in view of the intended use of the book by students being introduced to general practices relating to a major industry. There is an excellent bibliography of some six hundred references, many of which call attention to original research data. This provides the reader with easy access to a more detailed study on many of the subjects presented in this book. In this way, a vast number of subjects pertinent to the source and use of fertilizers, and which would ordinarily require several volumes, are presented in this single volume. Detailed, technical description of the manufacture and use of fertilizers would be out of place in a book of this nature. The author has done an admirable job in avoiding such detail and the result is an outstanding work on the general subject of commercial fertilizers, their sources and their use.

JOHN O. HARDESTY

Fatty Acids, Their Chemistry and Physical Properties. By Klare S. Markley. Interscience Publishers, Inc., New York, 1947. X+668 pp. Price $10.00.

The purpose of this work, states the author, is "to bring together in an organized and readily accessible form as much as possible of the present accumulation of facts and data pertaining to the chemical reactions and physical properties of the fatty acids . . . " and thus "obviate many hours of searching the literature by the large and growing body of chemists, physicists, engineers, and technologists who are interested in the fatty acids and their numerous products and by-products."

The scope and emphasis of the book may be indicated to a certain degree by noting the number of pages devoted to the major divisions of the book, together with the names of the chapters under each division. The book is separated into six major divisions:

A. The Nature and History of Fats and Waxes, 9 pp. in one chapter.

B. Classification and Structure of the Fatty Acids, 64 pp., includes two chapters; Classification and Nomenclature, and Isomerism.

C. Physical Properties of the Fatty Acids, 166 pp., includes five chapters; Crystal Properties, Spectral Properties, Thermal Properties, Solubility of Fatty Acids and Solution Properties; and Properties of the Fatty Acids in the Liquid State.

D. Chemical Reactions of the Fatty Acids, 278 pp., includes eleven chapters; Salts of Fatty Acids, Esterification and Interesterification, Alkylation and Alkoxylation, Pyrolysis, Halogenation, Hydrogenation and Hydrogenolysis, Oxidation and Hydroxylation, Oxidation by Atmospheric Oxygen (Autooxidation), Biological Oxidation, Nitrogen Derivatives of Aliphatic Acids, and Sulfur Derivatives of the Fatty Acids.

E. Synthesis of Fatty Acids, 42 pp., includes two chapters; In Vitro Synthesis of Fatty Acids, and Biosynthesis of Fatty Acids.

F. Isolation and Identification of Fatty Acids, 57 pp., includes two chapters; Separation of Fatty Acids, and Identification of Individual Fatty Acids.

A cursory examination of the book discloses that experimental data such as physical constants, and that concepts such as those of crystal structure are well up-to-date, having been taken in many instances from the literature of the past ten years. It is to be regretted, though, that no reference was made to the work of A. W. Weitkamp on the acidic constituents of degras. Weitkamp claims to have isolated and identified thirty-two of the acidic constituents of degras, many of which had not previously been reported as occurring in nature (JACS, 67, 447 (1945)).

The history of the development of the chemistry of the fatty acids is very closely interwoven with the textual material throughout the book. The work is very well documented, a vast number of pertinent references to the original literature being given.

The text is profusely illustrated by a large number of tables, graphs, and diagrams, together with a few very helpful photographs.

The physical appearance and typographical set-up of the book are good; it is well printed on good paper, and durably bound. Except for an occasional word obviously misspelled, the book appears to be quite free from errors. At the back of the book is a useful author and subject index.

This book is a comprehensive survey of our present knowledge of the fatty acids and it will, no doubt, fill a long felt need in this field. As a reference book it will be found highly useful to chemists and other scientists working on fatty acids, or in related fields.

L. L. RAMSEY

Standard Methods for the Examination of Water and Sewage. Prepared, Approved, and Published Jointly by the American Public Health Association and the American Water Works Association. Publication Office American Public Health Association, New York 19, N.Y. Ninth Edition, 1946, XVII+286 pages. Illustrations. Cloth. Price $4.00.

This edition of one of the best known and most widely distributed laboratory manuals used by water and sewage control technicians, reflects the fine editorial work of Dr. Symons, as well as the activities of the nine cooperating and coordinating committees led by Maxcy, Pirnie, Buswell, Hitchens, Mallmann, Calvert, Black, Hatfield, and Gilcreas; and of the chairman of the joint editorial committee, Norton.

The reading matter on each page has been split in two columns which makes the directions much easier to follow and has been rearranged under fewer captions. It now consists of four parts instead of seven, as follows: Examination of water—physical and chemical; Sewage, Sewage Effluents, Industrial Wastes, Polluted Waters, Sludges and Muds; Microscopical Examination of Water, Sewage Sludge, and Bottom Sediments; and Bacteriological Examination of Water. The two appendices deal with Non-Standard Methods, and with Chemicals and Reagents.

It would be impracticable to refer even briefly to the many deletions, additions, and revisions represented by this editio .. One example must suffice. Under "Iron," the two introductory paragraphs have been omitted as such, and "Gravimetric," "Colorimetric-Bypyridine," and "Colorimetric-Phenanthroline" methods have been added. The potassium ferricyanide method for ferrous iron has been deleted. The bibliography for this subject has been increased from two to eight references.

The manual has been immensely improved, and the laboratory worker has a tool in this edition which will greatly increase his knowledge and efficiency. Access to the book is an actual necessity for laboratory workers concerned with the analysis of water and sewage.

J. W. SALE

The Water Soluble Gums. By C. L. Mantell, Ph.D., Reinhold Publishing Company, New York. 1947, 6×9 inches, V+258 pages of text, and a 7-page table "Native Designation of Gums." Price $6.00.

The author's aim in this book, as stated in the preface "is an attempt to coordinate information relating to the water-dispersible products derived largely from a wide variety of plants and known as gums." This aim is only partly fulfilled.

Chapter 1, Classification and Chemistry of the Gums, exemplifies the author's tendency toward brevity. The entire chapter comprises 18 pages; 4 pages, plus a flow sheet, is devoted to classification, leaving 13 pages for the discussion of general chemistry of gums. Physical chemistry of gums, an important subject, is disposed of in six pages. Then follows six chapters on individual gum families, such as acacia, India gums, Astralgus, and seaweed gums. These include much interesting detail on gathering and purifying these products. Considerable space is given in these chapters to their physical and chemical properties. The chapter on acacia gums includes an interesting discussion, the work of deJong and co-workers on "coazervation," or the effects produced by mixing two hydrophilic colloids of opposite electric charge. The chapter on seaweed colloids is particularly informative, due in part to the fact that the producers of agar agar and algin employ modern methods of manufacture and plant control. Excellent flow-sheets of the manufacturing processes are given.

Chapter 8, discussing the modified celluloses, industrial competitors of natural gums, is inadequate, considering the many uses for which these synthetic "gums" are recommended. Only four literature references are given, three dated before 1937. The American literature is entirely neglected. The two chapters on modified starches (dextrins), and on hydrophilic proteins (gelatins, etc.), are similarly brief, the author stating they are adequately treated in existing monographs. Pectin is not discussed.

Industrial uses of gums are disposed of in four chapters, totalling 41 pages. Here again the author seems to have neglected modern research, 100 of the 136 references listed being before 1940. 73 citations refer to patents. As an example, none of the work of Sommer, at Wisconsin, or Mack at Amherst, on stabilizers in ice cream is mentioned, although the statement is made "one of the largest uses of algin in the United States is as a stabilizer for ice cream."

Chapter 15 discusses "Specifications, Identification and Testing." This is too brief to be of practical value to a working chemist. Much important work is omitted. Work by Racicot and Ferguson on detection of gums in foods, and many A.O.A.C. referee reports on the same subject, are not given. The tests copied from *Methods of Analysis, A.O.A.C.* Fifth Ed., and the U. S. Pharmacopoeia XI, are both from obsolete editions. In fact, the XIII edition of the Pharmacopoeia is current. This is especially unfortunate since the Sixth Edition of the *Methods of Analysis* listed many tests and methods not included by the author. The same comment applies to methods published in *The Journal, A.O.A.C.* There is only one reference to articles from this journal dated after 1939. Many of the tests included in this chapter are abstracted too briefly to be of practical value as given. As an example, a method is given (p. 254), for the detection of agar in canned chicken. The paragraph ends "and the precipitate is treated like the like precipitate in the detection of agar in mayonnaise." The method for mayonnaise is given only by literature reference, and that indirectly in the discussion of gums in foods. Again (p. 256) is the statement "The methods for gums in cheese are applicable to vanilla ice cream." Nowhere in the book is a reference to a method for detection of gums in cheese.

One of the most valuable sections of the book is the last chapter. This is a table listing 179 native or trade names of gums, each with its corresponding botanical source, geographical origin and present day classification.

In general, the book is characterized by incomplete literature research. The only other book dealing specifically with gums known to this reviewer, "The Biochemistry of Cellulose, Polyuronides, Lignins, etc." by A. G. Norman (1937) is not mentioned. The many papers by Tseng, of Scripps Institute of Oceanography, University of California, on algin and agar are dismissed with two brief references. Other examples have been given. Mantell's book impresses this reviewer as being too technical for the industrialist or scientist. The author could well have added 100 pages or more without introducing any new subjects. The book is printed on a good quality paper, with easily-read type, and contains informative flow-sheets, graphs, and illustrations. The bibliography, while not complete, is representative, and contains the more important potent citations.

F. LESLIE HART

Cereal Laboratory Methods, Fifth Edition, 1947, XIV, pp. 341, illustrations. Cereal Chemistry Office, University Farm, St. Pail 1, Minn. $4.50.

This is essentially a "methods of analysis" book for particular use in cereal testing laboratories. The new edition has been revised and considerably expanded through the addition of new chapters on experimental malting, vitamin assay methods, and sanitary methods. A number of new methods have also been added in previously existing chapters: specifically the xylene partition and thiobarbituric acid methods as a replacement for the old phloroglucin method for pentosans, the determination of urea and ammoniacal nitrogen, the determination of egg solids in macaroni products, and the fat stability test. Likewise, a number of methods have been deleted: glutenin, proteins soluble in 70% ethyl alcohol, proteins soluble in 5% K_2SO_4 solution, proteins peptized by neutral normal salt solution, albuminoid nitrogen, buffer value of flour, water soluble protein precipitable by 40% alcohol, lipoids, lipoid phosphoric acid, detection of sesame oil, and the accelerated rancidity test. Many of the methods described are the methods adopted by the Association of Official Agricultural Chemists, and by the American Oil Chemists' Society.

A criticism is justified of the provision for sampling feed by the same procedure recommended for sampling flour, rather than by the feed sampling method of the *Methods of Analysis, A.O.A.C.*, and the Official Publication of the Association of American Feed Control Officials, Inc. The (identical) methods of the latter two organizations are generally used in feed enforcement activities.

The increased scope of the subject matter makes this a more valuable book than the previous edition, and it will be a useful addition to any laboratory working in the field of cereal chemistry.

V. E. MUNSEY

FIRST DAY

MONDAY—MORNING SESSION

REPORT ON ALCOHOLIC BEVERAGES

By J. W. Sale (U. S. Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

Recently, the writer's attention was called to the omission from the sixth edition of "Methods of Analysis, A.O.A.C." of the official method for alcohol in beer, based on the use of the immersion refractometer, which appeared in the fifth edition of the book, Chapter XIV, sec. **5(c)**, pages 150–1. This is an error which is being called to the attention of the Editorial Board. The reference to the tables should be changed to apply to the sixth edition, as sec. **14.5(c)** the present reference table being **44.24** and **44.25**.

Attention of the Associate Referee on Color in Beer is called to the fact that we now have two methods for the determination of color in beer, namely, **14.2**—Color Official, and the tentative procedure adopted last year and published in *This Journal*, **30**, p. 68. Consideration should be given to making this latter method official and to deleting the former one.

In a letter of February 12, 1947, Mr. Allan D. Dickson, Associate Referee on Methods for Testing Soluble Starches used in Diastatic Power Determination of Malt, advised that available methods were not in shape for collaborative study. Moreover, the procedures do not lend themselves very well to collaborative work. Under the circumstances it appears desirable to discontinue this associate refereeship until some fundamental work has been conducted on soluble starches. The subject can then be reopened and collaborative studies resumed.

RECOMMENDATIONS*

The recommendations of the Associate Referees which are incorporated in the individual reports meet with the approval of the writer and are repeated here.

Malt Beverages, Brewing Materials, and Allied Products:

It is recommended—

(1) That the official, first action, methods for malt (*This Journal*, **30**, p. 55) be adopted as official, final action.

(2) That the official, first action, methods for beer (*This Journal*, **30**, p. 55) be adopted as official, final action.

(3) That the official, first action, methods for hops (*This Journal*, **30**, p. 56) be adopted as official, final action.

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* For report of Subcommittee D and action by the Association, see *This Journal*, **31**, 56 (1948).

(4) That the official, first action, methods for brewing sugars and sirups, (*This Journal,* **30,** p. 56), be adopted as official (final action).

(5) That the official, first action, methods for wort (*This Journal,* **30,** p. 56) be adopted as official (final action).

(6) That study of methods for determination of essential oil in hops be continued.

(7) That the study of the tentative method for color (*This Journal,* **30,** 68, 1947), and the photoelectric beer color evaluation, as well as work on beer turbidity methods, be continued.

(8) That the direct (non-ashing) orthophenanthroline method described in Proceedings of the Eleventh Annual Meeting of the American Society of Brewing Chemists, pages 32 and 37, for the determination of iron in beer, be studied collaboratively.

(9) That study of methods for testing soluble starches used in diastatic power determination of malt be discontinued.

(10) That study of methods for determination of total solids and yeast solids be continued in accordance with this year's report of the Associate Referee.

(11) That the Milos test for caramel, **14.35**, be deleted, first action.

(12) That the Mathers test for caramel, described in this year's report of the Associate Referee on Wine, be studied collaboratively with respect to its application to beer.

(13) That the study of carbon dioxide in beer be continued.

(14) That the official modified Denigés' method for methanol, section **16.25**, be studied together with the directions in **39.161–39.162**, to bring about uniformity in these procedures.

Wines:

It is recommended—

(1) That study of the spectrophotometric examination of wines be discontinued.

(2) That chromatographic studies in wines be continued.

(3) That study of methods for methanol in wines and distilled liquors be continued.

(4) That the official Milos test for caramel (**15.38**) be deleted (first action).

(5) That the Mathers test, as described in the report of the Associate Referee this year, be adopted as official, first action.

(6) That the tentative confirmatory test for caramel (**15.39**) be modified as described in the report of the Associate Referee for this year.

Distilled Liquors:

It is recommended—

(1) That the study of the obscuration method for determining the true proof of blended spirits be continued.

(2) That the study of methods of analysis with reference to the aging or maturing of whiskey in laminated (plywood) barrels be continued.

(3) That method **16.46 (b)** be changed as recommended by the Associate Referee in this year's report and that study of cordials and liqueurs be continued.

(4) That the modified Marsh test **16.39**, and the Milos test, official, **16.41** for caramel, be deleted (first action).

(5) That the Fulton test for caramel, described in this year's report of the Associate Referee, be adopted as tentative, for distilled liquors and for cordials and liqueurs.

(6) That the Mathers test for caramel, described in this year's report of the Associate Referee, be adopted as official (first action) for Distilled Liquors.

(7) That the Mathers test for caramel, described in this year's report of the Associate Referee, be adopted as official (first action) for cordials and liqueurs, and that it be included by reference in the chapter on cordials and liqueurs in **16.60**, in place of the modified Marsh test, **16.39**, and the Milos test, **15.38**.

(8) That an Associate Referee be appointed to study a rapid colorimetric method for fusel oil.

(9) That the method of fusel oil, **16.22** (p. 196) be further studied.

No reports were given on malt, on diastatic activity and soluble starches, or on hops.

REPORT ON FERMENTABLE EXTRACT IN BREWING SUGARS AND SYRUPS

By Philip P. Gray (Wallerstein Laboratories, New York, N. Y.), *Associate Referee*

The last report of the Associate Referee[1] recommended that the rapid method for determination of fermentable extract in brewing sugars and syrups be adopted as tentative. Since that time further collaborative studies of the method have been carried out by the subcommittee on sugars and dextrins of the American Society of Brewing Chemists under the chairmanship of the Associate Referee. The results of these collaborative studies were presented at the meeting of the A.S.B.C. in May 1945 and are embodied in the 1945 report of the Associate Referee on Brewing Sugars and Syrups[2] which recommended that both the regular method and the rapid method for determining Fermentable Extract be adopted as official-first action. The 1946 report of the Associate Referee on Brewing Sugars and Syrups[3] recommended further that both methods be adopted as official, final action.

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[1] Gray, Philip P., *This Journal*, **28**, 443 (1945).
[2] Laufer, Stephen, *ibid.*, **29**, 285 (1946).

It is therefore recommended* that further work on Fermentable Extract in Brewing Sugars and Syrups be discontinued.

REPORT ON INORGANIC ELEMENTS IN BEER

By G. H. Bendix (Continental Can Company, Inc., Chicago, Ill.), *Associate Referee*

IRON

Collaborative work on the methods for the determination of iron in beer has not been carried out since 1941 at which time reports were submitted by L. E. Clifcorn (3) describing a procedure involving the wet ashing of 25 grams of beer with nitric and perchloric acids, after which the red thiocyanate complex was developed in an acid methylcellosolve medium. In 1941 Clifcorn recommended this procedure be adopted as tentative.

In 1945 Bendix reported on the results of a survey which indicated a definite trend away from the use of thiocyanate for the determination of traces of iron. A preference for wet ashing techniques in place of dry ashing was also reported, and in addition it was mentioned that the determination of iron in beer had been carried out by Gray and Stone (4) without destruction of organic matter.

The method of Gray and Stone, which used $\alpha\alpha'$ dipyridyl and dispensed with the ashing step, was exceedingly simple and rapid, but it did not immediately receive general acceptance, probably because in 1938 photoelectric colorimetry was in its infancy. Gray and Stone reported that iron in beer exists in the ferrous state, and since dipyridyl responds only to ferrous iron, they were able to use it directly without an ashing treatment.

In 1946 Nissen (5) and Bendix (1) reported to the American Society of Brewing Chemists results obtained by a direct method developed by Nissen using ortho-phenanthroline. Nissen's method also differs from that of Gray and Stone in that hydroxylamine is added to assure iron in the ferrous condition. The method employs photoelectric colorimetry.

Tables 1 and 2 show the results as taken from Nissen's report (5). It may be noted that excellent agreement is obtained both in the case of comparison to the ashing KCNS method and in the case of recoveries of added iron.

The Nissen method, with slight modifications, has been used extensively by the Associate Referee's laboratory and by others,* with complete confidence and satisfaction.

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[3] *Ibid.*, 30, 214 (1947).

* For report of Subcommittee D and action by the Association, see *This Journal*, 31, 56 (1948).

COPPER

In 1945 Bendix (2) reported on collaborative work describing the application of an all-dithizone method for the determination of copper in beer.

TABLE 1.—*Comparison of iron methods*

SAMPLES OF BEER		IRON IN P.P.M.	
NO.	RANGE—(Fe P.P.M.)	DIRECT ORTHOPHENANTHROLINE METHOD	ASHING KCNS METHOD
56 Samples	0.00–0.03	0.02	0.024
4 Samples	0.04–0.06	0.05	0.05
5 Samples	0.07–0.10	0.09	0.07
6 Samples	0.11–0.16	0.12	0.11
3 Samples	0.24–0.26	0.25	0.24
2 Samples	0.32–0.34	0.33	0.27
		0.44	0.35
		0.56	0.51
		0.59	0.53
		0.63	0.63
		0.76	0.74
		0.80	0.76
		0.86	0.88
		1.03	1.05

TABLE 2.—*Recoveries of added iron*

SAMPLE	IRON ADDED, P.P.M.	IRON FOUND P.P.M.	IRON RECOVERED, P.P.M.	IRON RECOVERED, PER CENT
1		0.03		
2	0.05	0.08	0.05	100
3	0.10	0.12	0.09	90
4	0.20	0.24	0.21	105
5	0.40	0.44	0.41	102
6	0.50	0.53	0.50	100
Ave.				99.5

While this method gave results which were not unsatisfactory, several improvements in all-dithizone methods have been devised and will be studied collaboratively by the Referee on metals in foods. The general procedure developed in this work should also be applicable to beer.

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* Private Communication from the American Can Company.

TIN

In the Referee's opinion no methods are available for the determination of tin in beer, which offer satisfactory promise for use as an A.O.A.C. referee method. Investigative work involving the use of the polarograph for this determination has been carried out in the Referee's laboratory, but at the present time progress has not been sufficient to warrant collaborative work on a polarographic method.

RECOMMENDATIONS*

It is recommended—

(1) That the direct (non-ashing) orthophenanthroline method be submitted for collaborative work.

(2) That collaborative work on the determination of copper be postponed pending the outcome of proposed work by the Referee on metals in foods.

(3) That no collaborative work be done on the determination of tin in beer until the development of a more promising method.

LITERATURE CITED

(1) Bendix, G. H., and Strodtz, N. H., Proceedings of the Eleventh Annual Meeting of the American Society of Brewing Chemists, p. 37.
(2) Bendix, G. H., *This Journal*, **25**, 456 (1945).
(3) Clifcorn, L. E., *Ibid.*, **25**, 277 (1942).
(4) Gray, Philip, and Stone, Irwin, *Ind. Eng. Chem., Anal. Ed.*, **10**, 415 (1938).
(5) Nissen, B. H., Proceedings of the Eleventh Annual Meeting of the American Society of Brewing Chemists, p. 32.

No report was made on brewing sugars, sirups, wort, spent grains, and yeast.

REPORT ON SOLIDS IN YEAST

By Robert I. Tenney (Wahl-Henius Institute, Chicago, Ill.), *Associate Referee*

Inaccuracies in reporting upon the solids content of yeast samples, especially those representing yeast removed from breweries, present several serious problems. Naturally, the financial consideration between the brewer and the processor is affected; but, more important, these figures are entered upon the brewers' records which are inspected from time to time by representatives of the Alcohol Tax Unit of the Treasury Department. A.T.U. Regulations (1) require that yeast removed from breweries shall contain not less than 15 per cent of solids. Furthermore, yeast slurries containing more than 12 per cent of solids are frequently too viscous to transfer through pipe lines and hoses properly. The obvious practical solution is to dilute with water, and this is done under conditions which seriously complicate the taking of truly representative samples. Any method proposed by this Society should recognize these difficulties and strive to yield results which can be reasonably duplicated by two or more laboratories that might be participating in any controversy arising about a yeast shipment. The Associate Referee was charged with continuing the investigation of methods for both total solids in yeast and for yeast solids.

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* For report of Subcommittee D, and action by the Association, see *This Journal*, **31**, 56 (1948).

The method currently given tentative status as **14.115**, for total solids, was adopted at the 1944 fall meeting and is one which has been in use by the American Society of Brewing Chemists since 1942. It appeared in that Society's published methods (2) after sufficient collaborative data had been obtained to justify its inclusion. Since that time collaborative study has been continued by members of the A.O.A.C. and the A.S.B.C. A portion of this work has been directed toward other methods and a portion toward the variations noted between laboratories.

The Associate Referee is greatly indebted to Fred A. Wilcox, of Wahl-Henius Institute, who as chairman of the Yeast-Analytical Committee within the American Society of Brewing Chemists, made much data available. He is also indebted to the following collaborators and conferees, whose cooperation is gratefully acknowledged.

1. Carroll A. Dayharsh, Jacob Ruppert Brewery, New York, N. Y.
2. Clarence Estes, Anheuser-Busch, Inc., St. Louis, Mo.
3. Stephen Laufer, Schwarz Laboratories, New York, N. Y.
4. George W. Kirby, Fleischman Laboratories, New York, N. Y.
5. Eugene V. Nay, St. Louis Brewers Yeast Corp., St. Louis, Mo.
6. Joern Olshausen, J. E. Siebel Sons Company, Chicago, Ill.
7. F. O. Rickers, F. & M. Schaefer Brewing Co., Brooklyn, N. Y.

Some of the variations noted in previous collaborative work have been thought to be due to autolysis of the samples. To avoid this possibility and yet permit comparison of the method between widely separated laboratories, a large sample was divided and the separate portions autoclaved at 15 pounds for 30 minutes. Results obtained by five laboratories using method **14.115** are shown in Table 1.

From this table, the method appears capable of giving close agreement between laboratories. The maximum deviation between different laboratories in this case is equal to the greatest difference in duplicates for one collaborator.

Earlier collaborative attempts between widely separated laboratories did not give as consistent results. While a part of this disagreement may be blamed upon lack of familiarity with the new procedure and failure to follow it precisely, it is now thought that sampling had as great an influence as any other factor.

Yeast slurries must be recognized as difficult materials to sample. They settle out fairly rapidly and chemical changes due to continued enzyme action are bound to occur in transit unless special precautions are taken. The significance of autolysis as it affects both total solids and yeast solids is currently being studied by a committee of the American Society of Brewing Chemists.

Carroll Dayharsh has proposed a toluene distillation type of procedure for determining total solids in yeast and has submitted a good many figures to the Associate Referee in support of his views. The speed with which such an analysis may be conducted is certainly a point in its favor, though results differing from those of the tentative A.O.A.C. method may be anticipated. In general, it may be said to agree more closely to those methods in which some of the solubles are washed from the yeast with water before drying. The method has not yet been subjected to collaborative study, but it will be included in future A.S.B.C. work.

TABLE 1.—*Total solids in autoclaved yeast slurry*

COLLABORATOR	RESULTS		DIFFERENCE	AVERAGE
	Per cent	*Per cent*	*Per cent*	*Per cent*
I	22.2	22.1	0.1	22.15
II	23.1	22.9	0.2	23.0
III	22.9	22.4	0.5	22.65
IV	22.3		—	22.3
V	22.5		—	22.5

Lowest result, 22.1%, high 23.1%
Overall average, 22.5%
Maximum deviation, 0.5%

The situation with respect to yeast solids is entirely different. Here we approach a question of definition not unlike that involved in crude fiber determinations. The Associate Referee holds that total solids should, by definition, include all nonvolatile substances in the slurry except obvious extraneous matter known to be not representative of the whole. Any method which washes the yeast before drying is an attempt to remove a portion of these total solids, and hence is an approach to yeast solids.

To date, no method of washing out a portion of the slurry selectively has proven capable of interlaboratory agreement. The report to the American Society of Brewing Chemists last May (3) pointed out that the sediment from a brewery fermenter commonly termed yeast consisted of a mixture of unbroken yeast cells, broken yeast cells with contents partially or totally spilled, beer, hop resins, separated proteins, and gums. The question arises, "How much of this mixture is yeast?" The effects of several solvents was compared but no suggested method was developed.

The same collaborators shown in Table 1 also determined yeast solids by a water washing technique on the autoclaved yeast. The results varied from a low of 7.839 per cent to a high of 16.9 per cent although interlaboratory variations did not exceed 0.4 per cent. Therefore a satisfactory procedure for determining yeast solids is as lacking as is a definition to which all will agree.

RECOMMENDATIONS*

In view of the described situations it is recommended:

(1) That method **14.115** for total solids in yeast be continued in tentative status pending the outcome of further study.

(2) That the influence of autolysis upon this determination be studied in detail.

(3) That a non-miscible solvent distillation method be studied as a possible alternate.

(4) That study be continued seeking a definition and method for determination of yeast solids.

(5) That the attention of laboratories be called to the necessity of taking truly representative samples from yeast slurries by inclusion in the method of a paragraph substantially as follows:

SAMPLING

Yeast slurries tend to settle rather rapidly and are frequently quite viscous. These facts must be kept in mind when drawing samples from tanks and other large containers. While satisfactory aliquots may be obtained for laboratory purposes from small samples after thorough agitation mere agitation is not usually sufficient to insure a uniform mix in a tank or vat. In taking the sample a device which will permit samples to be drawn from several portions and depths of the tank should be employed. A satisfactory device is a watertight trier such as is used in grain sampling. Or a continuous sampling device may be attached to a hose or pipe line used in transferring the yeast from tank to transport car or truck tank and this composite thoroughly mixed before a smaller sample is withdrawn.

REFERENCES

(1) Article 21, Section 192. 149, Regulation 18, Alcohol Tax Unit, Treasury Department (1940).

(2) *Book of Methods*, Yeast I. American Society of Brewing Chemists (1944).

(3) TENNEY, *et al.*, *Proceedings Annual Meeting*, A.S.B.C., 1947.

No reports were given on beer, or on acidity and *p*H of beer.

No reports were given on color and turbidity in beer, or carbon dioxide in beer.

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* For report of Subcommittee D and action by the Association, see *This Journal*, 31, 56 (1948).

REPORT ON CARAMEL IN WINES AND DISTILLED LIQUORS

By PETER VALAER (Bureau of Internal Revenue, Washington 25, D. C.), *Associate Referee*

Chemical work was initiated during the year 1945 for the purpose of improving the accuracy and making more concise the Chemical Methods for the Qualitative Detection of Caramel in wines and in spirituous liquors. The new procedures which will be set forth in detail below are applicable for the qualitative indication of caramel in any fluid substance, aqueous or alcoholic.

The Milos test designed for the qualitative determination of caramel is at present an A.O.A.C. official method, made so after the report of the collaborators and recommended by the Associate Referee and presented at the annual A.O.A.C. meeting in October, 1944[1] and at the same time a supporting test was adopted which became "Confirmatory test-tentative." Reference was also made in Chapter 14 on "Malt Beverages" etc., to "Caramel," Milos test—official, p. 158. In the same report it was recommended that cyclohexanol test be also accepted, and it is now included with the Marsh test in Chapter 16 "Distilled Liquors." All these appeared for the first time in the A.O.A.C. Methods of Analysis, 6th Edition, 1945. Although the Milos procedure, as it appears in the above volume (page 188), and its attendant "confirmatory-tentative," has given useful service, the collaborators and the Associate Referee feel, however, that the Milos test could be advantageously replaced by a shorter and still more conclusive method, accompanied by one good confirmatory test for caramel, instead of the present number of small tests.

This new proposed method devised by Alex P. Mathers, Bureau of Internal Revenue, Washington, D. C., and referred to as the "Mather's Test," is recommended as an official substitute for the present Milos test, to be sustained by the present confirmatory-tentative (p. 189), this last being only slightly changed. The additional tests, such as paraldehyde and the others which accompany this confirmatory test, are omitted in favor of the test using 2, 4-dinitrophenylhydrazine reagent.

The details of the methods are described in *This Journal*, **31**, 76, 1948, under "Changes in Methods of Analysis."

These changes have been justified after a great deal of analytical work during the past two years which tended to prove that the new procedures are more conclusive, and saving in time and page coverage; and it is the consensus of opinion of the collaborators that they are, on the whole, better than those caramel methods which now appear in the 6th Edition (1945) of Methods of Analysis.

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[1] *This Journal*, **28**, 467 (1945).

In order to make a beginning for this investigation and to support any recommendations made for the proposed tests with collaborative results by as many chemists as possible, in 1946 and 1947, eight large bottles each containing several liters of wine and whiskey were prepared and analyzed as thoroughly as possible with existing caramel methods, the basic material in each case being caramel free. Into two of these batches one of wine and one of whiskey was incorporated a small amount of caramel coloring, the color of the basic material due to natural ingredients was much darker than the color produced by the caramel used.

Each collaborator or collaborating laboratory was sent a 4 fluid-ounce sample of each of the eight prepared batches consisting of four wines and four whiskies.

The letter of transmittal accompanying the eight samples sent to each collaborator requested that the samples be analyzed for caramel coloring and that report be made as to which method was considered best.

Suggestions and comments were requested as to how the methods may be improved and how these methods compare with other methods used for the detection of the presence of caramel coloring matter in beverage products.

The tests described in this report have been thoroughly tried out with all the difficult coloring substances encountered over several years, including those which interfere with the usual caramel tests and those which might possibly be mistaken for caramel.

Key to Unknown Samples

Sample		
1	W-1	*Sample W-1* consisted of odds and ends of old commercial blackberry wines and some other old fruit wines, to which was added a small amount of commercial caramel coloring.
2	W-2	*Sample W-2* consisted of old darkened miscellaneous fruit wines sweetened with very old and dark grape concentrate, to which was added very dark pokeberry juice and very darkest brown sugar (raw).
3	W-3	*Sample W-3* consisted of very old plum wine which had become almost black with age.
4	W-4	*Sample W-4* consisted of old plum wine, very old Elderberry wine, molasses, and the darkest specimens of raw Cuban sugar.
5	S-1	*Sample S-1* consisted of straight aged whiskey, whose natural color was heavily fortified with toasted white oak chips.
6	S-2	*Sample S-2* consisted of straight-aged whiskey, heavily colored with a strong hydro-alcoholic infusion of white oak chips.
7	S-3	*Sample S-3* consisted of straight aged whiskey, further colored with an infusion of uncharred chips and a small amount of caramel.
8	S-4	*Sample S-4* consisted of straight aged whiskey, further colored with an infusion of charred chips, uncharred chips, molasses, brown (raw) sugar, and an infusion of peat-dried barley malt.

All of the above samples gave positive indications with Marsh and Williams reagents, although only 2 actually contained caramel.

COLLABORATORS

The following chemists collaborated in this investigation:

Romig, Milos, and others, in Alcohol-Tax, New York Laboratory.
Riley and Watson, in Alcohol-Tax, Louisville, Laboratory.
Quillen in Alcohol-Tax, Baltimore Laboratory.
Fonner, Carr, and others in Alcohol-Tax, Chicago Laboratory.
Nealon and Forrest, in Alcohol-Tax, Detroit Laboratory.
Mallory, Love, and others, in Alcohol-Tax, San Francisco Laboratory.
Holman in Alcohol-Tax, Atlanta Laboratory.
Ringstrom in Alcohol-Tax, Seattle Laboratory.
Morawski and others, in Alcohol-Tax, Boston Laboratory.
Mathers, Blaisdell, Burritt, and Valaer, in Alcohol-Tax, Washington Laboratory.
John Wilson, Washington, Food and Drug Laboratory.
Loughrey, Boston, Food and Drug Laboratory.

Since all the collaborators found caramel in W-1 (wine) and in S-3 (spirits) and did not find caramel in any of the six other samples by the two proposed methods, it is pointless to present their findings in any usual tabulated form. Other tests were made by other methods and excesses were reported, which is not surprising when we consider the unusually difficult interfering substances that were incorporated in these unknown samples.

COMMENTS OF COLLABORATORS

Although opinions and comments were requested a number of collaborators merely reported without further statements; however some of them commented to the following extent:

Nealon and Forrest, stated that "both methods were rapid and excellent for the separation of caramel coloring matter from wine and spirits; there seems to be little, if any, foreign matter precipitated with the caramel, which renders the confirmatory tests more positive. However, since the Mathers test is somewhat more rapid and neutralization of the sample is unnecessary, it is to be preferred over the A.O.A.C. Method."

Blakely and Clapp were of the opinion that a stronger indication of caramel was obtained with Mathers test than with the A.O.A.C. tentative-confirmatory; there was some objection expressed to the washing required by the latter.

Fonner and Carr found "both methods rapid and simple, with the A.O.A.C. procedure satisfactory except requiring more washings."

Ringstrom found "both methods worked very well on the samples and both gave clean clear-cut results; however the Mathers method required less washing than the A.O.A.C. method to remove natural coloring from the wine samples, and consequently it is the shortest good method for determining the presence of caramel coloring."

J. H. Loughrey "liked the $Zn(OH)_2$ precipitation; even though it brings down only a small % (about 14%) of the caramel. I particularly prefer the 2,4-di-nitro-phenylhydrazine as the precipitating test; it is much easier to prepare and keep in solution; the precipitate with caramel is quite definite."

Quillen stated, "I am very much impressed with the Mathers method, especially its simplicity, the ease and rapidity with which the test can be performed."

Morawski and Forbes were of the opinion that "the Mathers and A.O.A.C. methods are equally efficient. Many tests were carried out with both methods taking Bottled in Bond whiskey of a tintometer reading of 16, and adding caramel to portions, thus increasing the total color to 17 and 18. This was done in order to satisfy ourselves as to the sensitiveness of the methods." "Both methods are good but we are inclined to use the Mathers test because of the smoother operation and less time involved to complete the tests."

Holman, besides analyzing the samples, made other experiments by adding caramel to various samples and "in each instance the results were very satisfactory."

Riley and Watson, "In the Mathers test, instructions call for the addition of 50 ml or more of alcohol to the 10 ml sample and to the residue dissolved in 10 ml of water. The Babcock bottles in the District laboratory have a total capacity of 50 ml, which would permit the addition of only 40 ml of alcohol. Approximately the same alcoholic content could be obtained by dissolving the residue in the centrifuge bottle in 8 ml of water, instead of 10 ml, and adding about 40 ml of alcohol, to make a total volume of not over 50 ml. (So far we have found the Babcock cream bottle satisfactory; a larger centrifuge bottle is sometimes used.)

Mathers method states: "Repeat this process until the upper alcoholic layer is quite clear and colorless." Does this mean completely or entirely clear and free from all color and opalescence? (The upper solution should be without color—opalescence is of no consequence.)

On some samples the gelatinous residue by the Mathers method was not firmly held in the centrifuge bottle and would break loose when the supernatant liquid was decanted. Better results were obtained by inserting a small glass tube into the centrifuge bottle and removing most of the supernatant liquid by a slight vacuum or siphon action then decanting the small amount of supernatant liquid which remains. The failure to obtain a firmly packed residue in all cases may be due to inadequate speed of the centrifuge employed.

In the A.O.A.C. confirmatory test considerable KOH was required to neutralize sample W-3, which left little room for reagent and alcohol in the centrifuge bottle. Use of a stronger KOH solution on samples containing considerable acidity would reduce the final volume. Some difficulty was experienced in dissolving the residue first obtained in the A.O.A.C. confirmatory method. Very hot water and heating in boiling water plus shaking was required.

The Mathers test required less time than the A.O.A.C. confirmatory test, because less washing was required; there is no difficulty in dissolving the residue and it is not necessary to neutralize the sample before starting the test."

Practically all the collaborators believed that the new reagent (2,4-dinitro-phenyl-hydrazine) was more satisfactory in operation than the older phenyl-hydrazine reagent (p. 189) and find this new reagent much more stable, keeping apparently indefinitely.

The unusual number of chemists that participated in this collaborative investigation were practically all of the opinion that the Mathers method was one of the quickest and best methods yet devised for caramel detection, and when sustained by the present confirmatory test tentative (p. 189), and the final caramel solutions from each method confirmed by the 2,4-dinitro-phenyl-hydrazine reagent, it would produce one of the strongest combinations of tests for caramel so far obtained.

In Chapter 16 on "Distilled Liquors," the Marsh test-tentative for artificial color (**16.38**) has been an A.O.A.C. procedure for a number of years and has been quite useful in that it shows the presence of artificial color, caramel, sometimes coal tar, and other coloring matter.

The official modified Marsh test **16.39** (8) was originally designed to determine whether a positive reaction given by Marsh reagent was caramel or was given by the presence of coloring matter from raw or untoasted white oak chips, or due to long storage in plain uncharred barrels. This can now be replaced by the simpler test, cyclohexanol reagent, and Fulton's reagent, and the Mathers test or the A.O.A.C. tentative-confirmatory, or all of them, to make assurance doubly sure.

A cross reference to these last two tests would be sufficient in the Distilled Spirits chapter in the same manner as the Milos test-official is referred to in the 6th Edition, p. 202.

It is best, in applying the Mathers test to lightly colored spirituous liquors such as imported blended Scotch whiskies, to use 50 ml of the sample and to reduce its volume to about 10 to 12 ml on a steam bath, hastened by aid of a current of air (electric fan) and filter it; and on this approximately 10 ml continue with the Mathers or A.O.A.C. confirmatory tests, as outlined above.

The Cyclo-hexanol test-tentative (**16.40**), has proved its worth and should be retained; but the Fulton test, which up to this time has not sought entrance into the A.O.A.C. Methods of Analysis should be placed next to the cyclohexanol test as a supporting or an extra check.

The Fulton test was devised principally for the same purpose as the cyclohexanol test, to distinguish between caramel and uncharred white oak color in distilled spirits, since uncharred white oak gives a color in the lower layer of the Marsh test and the Williams test.

The Williams test, extensively used by A.T.U. Junior inspectors, was devised by John F. Williams (U.S. Customs service) and designed to show not only the presence of artificial color in commercial spirituous liquors, but also gives a fairly accurate determination of proof. The Williams reagent consists of 70 parts of normal amyl alcohol (Pentasol); 28 parts of toluol; and 2 parts of dilute HCl (conc. HCl dil. 1 to 10). In testing for color in spirits it is used in the same manner as the Marsh reagent.

It is true that at present not a great deal of spirituous liquor is colored or "quick-aged" with uncharred chips. However, blended Scotch whiskey, Scotch grain spirits, and Scotch malt whiskey is usually long aged in plain barrels, if the barrels are previously unused or not much used, more or less color will be extracted by the whiskey over the traditionally long years of storage, some of which will show up in the lower layer of Marsh and Williams reagent but not in the lower layers of Cyclohexanol-tentative test (**16.40**, p. 202), or in the Fulton reagent, which is herewith proposed as an additional test for caramel and other coloring matter.

Caramel is added to Scotch whiskey only when it is necessary to produce a certain standard depth of color, for the sake of uniformity of this product.

The formula for the Fulton's Acetone Reagent is as follows:

FULTON TEST

REAGENTS

(a) *Acetone reagent.*—Acetone, 500 ml; amyl alcohol, 200 ml; ethyl acetate, 200 ml; syrupy H_3PO_4, 50 ml; water, 50 ml.
(b) Sodium di-hydrogen phosphate.—NaH_2PO_4, 25 g; water, 100 ml.

DETERMINATION

To ca 5 ml of spirits add 10 ml of the acetone reagent and 3 ml of the NaH_2PO_4 soln, shake and let stand. Color in the lower layer indicates caramel.

This reagent does not register a positive test with vegetable matter, uncharred chip color, charred or toasted chip color; but with some coal tar dyes occasionally used for coloring whiskey there may be a weak positive indication.

It has been found that Mathers test is better for some kinds of samples and the tentative-confirmatory test gives better results for other kinds of material, so it is recommended that both be applied to any sample in question, followed by the 2, 4-di-nitro-phenyl-hydrazine reagent confirmatory on any final brown solution obtained in both tests.

Substances like very old fruit wines which are unusually difficult to remove can be conclusively diagnosed by taking a much larger sample and condensing it to 10 ml or less. A large quantity of the sample does not increase the indication; except that when caramel is present the strength of the test is correspondingly increased with the amount of the sample taken.

RECOMMENDATIONS*

It is recommended—

Wines:

(1) That the official Milos test for caramel **(15.38)** be deleted, first action.

(2) That the Mathers test for caramel as described (*This Journal*, **31**, 76, 1948), be adopted as official, first action.

(3) That the tentative confirmatory test for caramel **(15.39)** be modified as described on page 77, *loc. cit.*

Distilled liquors:

(4) That method **16.46(b)** be changed as recommended in the Associate Referee's report.

(5) That the official modified Marsh test and the official Milos test, **16.39** and **16.41**, for caramel be deleted, first action.

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* For report of Subcommittee D and action by the Association, see *This Journal*, **31**, 57 (1948).

(6) That the Fulton test for caramel, as described by the Associate Referee, be adopted as tentative for distilled liquors and for cordials and liqueurs.

(7) That the Mathers test for caramel, described in this year's report of the Associate Referee, be adopted as official, first action, for distilled liquors.

(8) That the Mathers test for caramel, as described by the Associate Referee, be adopted as official, first action, for cordials and liqueurs, and that it be included by reference in the chapter on cordials and liqueurs in sec. **16.60**, in place of the modified Marsh test, **16.39**, and the Milos test, **15.38**.

REPORT ON FUSEL OIL IN DISTILLED SPIRITS

By G. F. BEYER (Bureau of Internal Revenue, Washington 25, D. C.), *Associate Referee*

In view of the lengthy and time-consuming procedure of the fusel oil determination, experiments were made with a view toward shortening the method without interfering with its accuracy.

The procedure suggested is as follows:

To 50 ml of the sample add 10–12 ml of 0.5 *N* NaOH, add 60–70 ml dist. water and distil slowly until ca 75 ml have been received. Add ca 100 ml H_2O and saturate with NaCl. Ext. this salt soln 4 times with CCl_4, using 40, 30, 20, and 10 ml, respectively, and wash the CCl_4 3 times with sat. NaCl soln and twice with satd. Na_2SO_4 soln. Transfer the CCl_4 to Erlenmeyer flask containing 50 ml of the oxidizing soln and oxidize for 4 hrs. under a reflux condenser. Ground glass connections in all cases should be used and no grease.

Add 15–20 grains (granules) of #20 carborundum and 50 ml distd. water thru the condenser and distil until only ca 50 ml remains. Add 50 ml of H_2O and distil again until 35–50 ml are left.

Experiments have also shown that certain losses occur; that is, there is a 3–5 per cent loss in the CCl_4 up to the time of oxidation, and then it seems that a small amount of the fusel oil is washed out with the salt solution.

This procedure was carried out in parallel with that described in the *Methods of Analysis, A.O.A.C.*, and the results agreed with each other within the limits of experimental error.

In view of the results obtained, therefore, it is recommended* that a further study be made with a view toward shortening the method, and also of determining how to prevent the loss during the washing of the CCl_4 with saturated salt solution.

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* For report of Subcommittee D and action by the Association, see *This Journal*, 31, 57 (1948).

No report was given on spectrophotometric examination of wines and distilled spirits or on chromatographic absorption of wines.

No report was given on methanol in wines and distilled liquors.

REPORT ON CORDIALS AND LIQUEURS

By John B. Wilson (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

The composition of various cordials and liqueurs varies widely. As defined by the Federal Alcohol Administration such products are "obtained by mixing or redistilling neutral spirits, brandy, gin, or other distilled spirits with or over fruits, flowers, plants, or pure juices therefrom, or other natural flavoring materials, or with extracts derived from infusions, percolations, or maceration of such materials, and to which sugar or dextrose or both have been added in an amount not less than 2½ per cent by weight of the finished product. Synthetic or imitation flavoring materials shall not be included." (Regulations 5, Art. II, Sec. 21, Class 6, page 8). Alcohol in such products may vary from about 25 to 70 per cent and the sugar content from the lower limit of 2.5 per cent imposed under the law, to 50 per cent or even more, as found in "Surfine" French liqueurs. It cannot be assumed that the coefficient of expansion of such mixtures is the same as that of a water solution containing the same relative volume of alcohol, and therefore it becomes obligatory to measure the sample at 15.56°C. and to make up the distillate to volume at 15.56°C., in order to obtain the correct percentage of alcohol by volume in cordials, unless the solids content approximates the minimum of 2.5 per cent.

RECOMMENDATIONS*

It is recommended:

(1) That **16.46(b)** (p. 203, *Methods of Analysis*, 1945) be changed to read as follows:

16.46(b) By Volume.—Proceed as directed under **16.6** except calibrate pycnometer used at 15.56°C. and measure sample at that temperature.

(2) That the study of cordials and liqueurs be continued.

REPORT ON FRUITS AND FRUIT PRODUCTS

By R. A. Osborn (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

RECOMMENDATIONS†

It is recommended:

(1) That work on methods for determining fruit and sugar content in frozen dessert fruits be continued.

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* For report of Subcommittee D and action by the Association, see *This Journal*, 31, 57 (1948).
† For report of Subcommittee D, and action by the Association, see *This Journal*, 31, 61 (1948).

(2) That further work be done on methods for the electrometric titration of acidity.

(3) That further collaborative study be made of methods of separating and determining fruit acids.

(4) That work on methods for the determination of water-insoluble solids be continued.

No reports were given on titration of acids, fruit acids, fruit and sugar in frozen fruits, or on water-insoluble solids.

REPORT ON CACAO PRODUCTS

By W. O. WINKLER (Food and Drug Administration, Federal Security Agency, Washington, D. C.) *Referee*

METHODS OF ANALYSIS

There are six projects in the analysis of cacao products which are being studied at the present time. These studies are on lecithin, maltose, lactose, cacao ingredients, pectic acid, and fat in refractory materials.

Lecithin.—A formal report was not made this year, but the Associate Referee made some comments. Last year collaborative work was carried on by the Associate Referee on a method proposed by him. Results were low, about 80 per cent in most cases. Supposing that the old lecithin was at fault, he bought a new supply this year. After careful mixing with chocolate at 40°C., the best recovery he could obtain was 81 per cent. Consequently, no samples were sent out this year. This type of problem has vexed us ever since the beginning of the work on lecithin. It has been reported that lecithin forms a loose combination with proteins, and this may be the reason for the difficulty in recovery.

Maltose and lactose in presence of other reducing sugars.—The Associate Referees on these materials are not ready to make formal reports but the Referee has knowledge that a considerable amount of spade work has been done on these projects.

Fat in refractory materials.—A report was submitted by the Associate Referee on this subject. He has modified the method suggested by Miss M. L. Offutt, the former Associate Referee, to use a Palkin extractor after the preliminary treatment, and has obtained very promising results by two collaborators. Further collaborative work is recommended.

Cacao Ingredient.—The present tentative method for estimating the cacao ingredient is based on the residue left after extracting fat, milk, and sugar solids with various solvents. The method is applicable to present standard cacao products, but where products contain other ingredients such as starch or cereal solids the method is not applicable. The constituents which are most characteristic of cacao are theobromine, cacao red, tannins, and modified tannins or phlobaphenes. The Referee has made a study to devise a method for determining the tannins, phlobaphenes, and most of the cacao red together; and a method has been developed which he believes is applicable to all types of products and the quantities of material determined are a considerable portion of the fat-free cacao. The details of the method proposed as a tentative procedure are published in "Tannins and Pigments," *This Journal* No. **31**, page 78 (1948). Preliminary results are given in Table 1.

TABLE 1.—*Per cent of cacao constituents*

Special reagent—40% formaldehyde solution

NO.	KIND OF COCAO BEAN	TANNIN AND PIGMENT PRECIPITATE
Sample		*per cent*[*]
(1)	Java	13.6
(2)	Unknown blend	13.38
(3)	⅔ Accra; ⅓ Bahia	12.25

[*] Percentage based on dry fat-free cacao.

Pectic Acid.—In the past some difficulty has been experienced in the filtration of the pectic acid precipitate. Even though the precipitate flocculated well and filtered well at first, it tended to become colloidal before washing was complete. This occurred at times regardless of the fact that saponification was made at temperatures below 20°C. The Referee has found that the washing can be done satisfactorily by using a 2 per cent salt solution in place of water and washing until free of acid. The precipitate is dried and weighed and is then ignited at about 600°C. in the muffle furnace, cooled, and reweighed. The salt is not affected at this temperature.

RECOMMENDATIONS[*]

It is recommended:

(1) That methods for the determination of lecithin in cacao products be further studied.

(2) That work on methods for maltose and lactose in the presence of other reducing sugars be continued.

(3) That the three procedures for fat in refractory beverage bases, described in the Associate Referee's report, be further studied.

(4) That further work be done on the determination of pectic acid in cacao products with particular reference to milk chocolate.

(5) That the method for tannins and pigments, as a means of estimating cacao ingredient, be adopted as tentative.

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[*] For report of Subcommittee D and action by the Association, see *This Journal*, **31**, 57 (1948).

No reports were given on lecithin, malt solids, pectic acid, cacao ingredients, or lactose; see Referee report, p. 186.

REPORT ON FAT IN CACAO PRODUCTS

By CARL B. STONE, (Food and Drug Administration, Federal Security Agency, Cincinnati, Ohio), *Associate Referee*

Committee D recommended at the last meeting that the work on the determination of fat in beverage bases and other refractory materials be continued.

The work this year covered methods reported in the *Journal*[1] by Miss Offutt, and several others. The General Referee suggested that the beverage material be thoroughly ground with sand and then placed in a continuous fat extractor. This was tried with a sample of malted milk flavored with chocolate and extracted with mixed ethers for a period of 16 hours. The duplicate determinations gave results of 1.78 per cent and 1.23 per cent.

Using the same beverage base the two methods suggested by Miss Offutt were then tried with the following results:

No.	*Product*	*Hillig Method* *Per cent*	*Roese-Gottlieb Method* *Per cent*
INV. 53-792 H	Chocolate Malted Milk	4.80	5.00
		5.25	5.04
			4.89

The Roese-Gottlieb method had a tendency to form an emulsion causing a difficult separation.

In view of this, the author thought it might be well to try using a Palkin extraction apparatus for the final removal of the fat. The Roese-Gottlieb method was used up to the point where the sample was transferred to an extraction set-up in place of the large separator suggested in the other method. The following results were secured on sample 53–792 H, after complete extraction with the mixed ether. The extracted fats were dried and reextracted in order to purify the fat material:

Analyst	*Fat %*
C. B. Stone	4.84
	4.91
H. C. Van Dame	4.88

In view of the limited results that have been secured, it is recommended* that all three procedures be studied further on several refractory beverage bases.

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[1] *This Journal*, 28, 482 (1945).

* For report of Subcommittee D and action by the Association, see *This Journal*, 31, 58 (1948).

REPORT ON SUGAR AND SUGAR PRODUCTS

By Carl F. Snyder (National Bureau of Standards, Washington 25, D. C.), *Referee*

The Associate Referees for the following subjects have submitted reports which will be presented at this meeting: Unfermented Reducing Substances in Molasses, Confectionery, Reducing Sugars, and Color and Turbidity in Sugar Products. In regard to the recommendations made in these reports, the Referee concurs. It is regretted that circumstances have prevented the Associate Referees on Honey and on Corn Sugar and Corn Sirup from presenting reports, but it is hoped that work in both of these fields may be carried out during the coming year.

THE INTERNATIONAL SUGAR SCALE

The Associate Referee, Dr. F. W. Zerban, of the New York Sugar Trade Laboratory, has called attention to the existence of uncertainties in the official method for estimating sucrose by direct polarization, **34.18**, and to the desirability of this Association officially adopting the International Sugar Scale. The method in question, employed chiefly in the analysis of raw sugars, was first introduced into the *Methods of Analysis* in the second edition in 1925, and has been included in all subsequent editions. Most of the changes that have been made during this period were of an editorial nature.

The method consists of the detailed directions for the direct polarization of raw sugars adopted by the International Commission for Uniform Methods of Sugar Analysis at its third session in Paris, 1900.[1] Included in these directions was the manner of verifying the scale of the saccharimeter by means of a solution of pure sucrose containing 26 grams in 100 ml soln. and polarized in a 200 mm tube at 20°C. The 100° point on the sugar scale was fixed as the reading of this so-called normal sucrose solution.

Following this meeting of the International Commission, Herzfeld and Schönrock,[2] on the basis of this definition of the sugar scale, standardized a number of quartz control plates on the saccharimeter and then measured their optical rotation in circular degrees on a polarimeter using sodium light. They reported that a quartz plate which read 100° on the saccharimeter with white light filtered through the bichromate cell specified by the Commission read 34.657 circular degrees on a polarimeter illuminated by sodium light. This version of the saccharimeter scale, which for all practical purposes had its 100° point fixed by the reading of a standard quartz plate, has been referred to as the Herzfeld-Schönrock scale. This scale superceded the existing Ventzke scale, whose basis of standardization was so vague that it led to uncertainty in the fixing of the 100° point.

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[1] *Z. Ver deut. Zucker-Ind.*, 50 (N. F. 37), 357 (1900); 63 (*N. F.* 50), 25 (1913); *J. Ind. Eng. Chem.* 5, 167 (1913); *This Journal*, 18, 162 (1935); National Bureau of Standards Circular C440, 1942, pp. 768, 774.

[2] *Z. Ver deut. Zucker-Ind.*, 50, 826 (1900); 54, 521 (1904).

An exhaustive study of the standardization of the saccharimeter scale was undertaken by Bates and Jackson,[3] whose preliminary report to the International Commission in 1912 showed evidence that the Herzfeld-Schönrock values were not quite correct. Their complete work, published in 1916, showed that the normal sucrose solution read 99.895 on instruments graduated on the basis of the Herzfeld-Schönrock values. This value was later verified by Balch and Hill[4] and by Zerban, Gamble, and Hardin,[4] who found values of 99.907 and 99.912, respectively. The average of the three determinations is 99.905. In addition to the saccharimetric values, Bates and Jackson determined the value for the optical rotation in circular degrees of the quartz control plate having a reading of 100 degrees on the saccharimeter.

The next meeting of the International Commission was held in 1932 in Amsterdam.[5] At this session the Commission adopted a standard scale for the saccharimeter, designating it the "International Sugar Scale" and the readings thereon as degrees sugar (°S). The rotation of the normal sucrose solution under the conditions defined by the Commission in 1900 was adopted as the primary basis of calibration of the 100° point on the scale, accepting the value 99.90° as the equivalent value on the Herzfeld-Schönrock scale. In addition, the following values were adopted, the normal quartz plate which reads 100°S on the saccharimeter has a rotation of 40.690 circular degrees for mercury light of wave length $\lambda = 5461\text{A}$ and a rotation of 34.620 circular degrees for sodium light of wave length $\lambda = 5892.5\text{A}$.

This action of the Commission virtually eliminated the uncertainty due to the existence of the several versions of the standardization of the sugar scale with the result that the new scale was accepted internationally. In view of these facts, it is essential that this Association officially adopt the International Sugar Scale, and a recommendation to that effect is presented.

Another question of importance considered at the Amsterdam meeting was the subject of the elimination of errors due to lead clarification in polarizing raw sugars. It was resolved to subject the matter to further study, using raw sugars from the various countries. The reports covering this collaborative work were presented at the ninth session in London in 1936.[6]

After a long discussion, the recommendation was adopted that in polarizing raw sugars on instruments calibrated on the International Sugar Scale clarification must be effected by dry basic lead acetate, the reagent being added after the sugar solution has been made to volume. In conformity with this it is recommended that official method **34.18** for raw sugars be amended accordingly.

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[3] *N. Bur. Standards Sci. Paper* No. 268, p. 67 (1916).
[4] *This Journal*, **12**, 106 (1929).
[5] *Int. Sugar Jour.*, **35**, 19 (1933).
[6] *Ibid.*, **39**, 328 (1937).

In incorporating the above recommendations it is deemed advisable to combine in a separate section of the method the directions for the verification of the saccharimeter scale as well as the values for the normal quartz plate.

The recommended changes in Methods **34.18** and **34.19** are given in detail in *This Journal,* **31,** (1948), together with other corrections, under "Changes in Methods of Analysis," on pages 110 and 111.

RECOMMENDATIONS*

It is recommended—

(1) That the Java method (*This Journal,* **29,** 242) for unfermented reducing substances in molasses be adopted as tentative, and that work be continued.

(2) That the study for the determination of moisture be continued.

(3) That study be continued on tables of density of sucrose solution at various temperatures.

(4) That the Zerban and Martin values for refractive indices of dextrose and invert sugar solutions (*This Journal,* **30,** 77 (1947)), be subjected to collaborative study.

(5) That the study of the applicability of electro-deposition to the direct quantitative determination of dextrin in honey and honeydew honey be discontinued.

(6) That the study of the characteristic properties of dextrins of honey and honeydew honey be discontinued.

(7) That the official method for the determination of free acid in honey, **34.99,** be studied collaboratively with a view to establishing the end point more accurately.

(8) That study be made of methods for the detection of adulterants in honey, particularly commercial syrups.

(9) That collaborative study of the method described in last year's report of the Associate Referee, for the determination of resinous glaze in confectionery be continued, with special reference to large amounts of lac.

(10) That study be continued on the determination of dextrose, maltose, and dextrins, by copper reduction methods in pure sugar mixtures.

(11) That the tentative methods, **34.133–34.155,** inclusive, be subjected to collaborative study.

(12) That the procedures in N.B.S. Circular C440, pp. 342–334, for measurement of transmission of solutions of commercial sugar products, be considered with a view to their future adoption as tentative methods.

(13) That the Somogyi Modification (*J. Biol. Chem.,* V. 160, p. 61, 1945) of the tentative micro method for reducing sugars (**34.63**) be studied.

(14) That changes in accordance with the International Sugar Scale be adopted as official, final (special action) in sections **34.18** and **34.19.**

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* For report of Subcommittee D and action of the Association, see *This Journal,* **31,** 61 (1948).

REPORT ON UNFERMENTED REDUCING SUBSTANCES IN MOLASSES

By F. W. Zerban (New York Sugar Trade Laboratory, Inc., New York, N. Y.), *Associate Referee*

In a series of reports on this subject[1] it was shown that the two methods of the Association generally used for the determination of reducing sugars, namely, those of Munson and Walker, and of Quisumbing and Thomas, do not give reliable results when applied to the determination of unfermentable reducing substances in molasses. It was suspected that the discrepancies might be due at least partly to differences in the Fleischmann's baker's yeast secured locally by the collaborators in various cities. In 1945[2] it was decided to exclude this factor by using dried distillery slop for the determination of the reducing substances, instead of first fermenting molasses with yeast and making the determinations on the residue. In this study the Quisumbing and Thomas method again gave unsatisfactory results, but the copper reduction procedure prescribed in the method of the Java Sugar Experiment Station[3] proved so promising that the entire method, comprising fermentation and copper reduction, was applied in this year's work to molasses.

Six collaborators expressed willingness to take part in this work and one sample each of cane blackstrap and of refiner's sirup was sent to them, with the directions as given in the following method:

UNFERMENTED REDUCING SUBSTANCES IN MOLASSES

Method of Java Sugar Experiment Station[4]

REAGENTS

(a) *Baker's yeast, free from starch.*—Fleischmann's, sold in packages of 1 lb. each by Standard Brands, Inc., was found suitable. It keeps fresh for a few days if kept in the refrigerator.

(b) *Neutral lead acetate soln.*—Dissolve 20 g $Pb(C_2H_3O_2)_2 \cdot 3H_2O$ in H_2O to 100 ml.

(c) *Anhydrous potassium oxalate.*

(d) *Soxhlet soln.* See section **34.33**(a) and (b).

(e) *Potassium iodide soln.*—Dissolve 20 g KI in H_2O to 100 ml.

(f) *Dilute sulfuric acid.*—5 volumes of water plus 1 volume of conc. H_2SO_4.

(g) *Standard thiosulfate soln.*—0.1 *N*.—See sections **43.28** and **43.29**.

FERMENTATION

Transfer 12 g of blackstrap molasses (or 8 g High-test molasses) to a 500-ml volumetric flask, using in all 75 ml of H_2O. Add 25 g of coarsely chopped, fresh baker's yeast and mix thoroughly with the molasses soln. Close the flask with a stopper provided with a delivery tube the other end of which dips about 1 cm below the surface of H_2O in a beaker; or use any other type of fermentation trap. Place the flask in a water bath kept at 30°C. and allow to ferment for at least 4 hours, shaking the flask from time to time. When fermentation is complete dilute with

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[1] *This Journal*, 23, 562 (1940); 24, 656 (1941); 26, 112 (1943).
[2] *This Journal*, 29, 242 (1946).
[3] "Methoden van Onderzoek," 6th ed. (1931), p. 365.
[4] "Methoden van Onderzoek bij de Java-Suikerindustrie," 6th ed. (1931), p. 365.

H_2O, clarify with 15 ml of neutral $Pb(C_2H_3O_2)_2$ soln, make to the mark at 20°C., add a teaspoonful of Filter-Cel, shake well and filter, discarding the first runnings. Delead the entire filtrate with ca 0.5 g of anhydrous $K_2C_2O_4$ and filter again with the aid of Filter-Cel. Test the filtrate for Pb. If necessary add more oxalate and refilter.

DETERMINATION

Transfer 25 ml of the final filtrate to a 250-ml Erlenmeyer flask, mix with 20 ml of combined Soxhlet soln, and wash the wall of the flask down with 5 ml of H_2O, making 50 ml in all. Add a few pieces of ignited pumice stone and place the flask on a wire gauze covered with an asbestos plate which has a hole in the center, slightly smaller than the bottom of the flask. As the source of heat use either a Bunsen burner or preferably an electric heater with temperature control. Heat to boiling in 3 min and boil gently for exactly 2 min longer. Close the flask immediately with a stopper provided with a Bunsen valve and cool quickly under the water tap to prevent reoxidation. Add 15 ml of KI soln and then 10 ml of dilute H_2SO_4. Titrate the liberated I at once with 0.1 *N* $Na_2S_2O_3$ soln, adding starch indicator toward the end of the titration.

Run a blank with 75 ml of H_2O instead of molasses soln, adding yeast, etc., as described. Deduct the titer of the sample from the titer of the blank, and find the mg invert sugar corresponding to the difference, from the table. The result, divided by 6 (in the case of High-Test molasses, by 4) gives directly the per cent of unfermentable reducing substances in the molasses, in terms of invert sugar.

Copper reduction, modified method (*II*).—In two other 25-ml aliquots of the final filtrate carry out the copper reduction exactly as described, but at the end of the 2-min boiling period filter at once thru a Gooch crucible with asbestos mat, or thru a fritted glass crucible, wash with hot water, and determine the copper in the precipitate, either by the thiosulfate method (*Methods of Analysis*, pp. 572, 573, sec. **34.41** and **34.42**), or by the permanganate method (*ibid.*, sec. **34.43** and **34.44**). Report the results of each experiment as milligrams metallic copper.

Milligrams of Invert Sugar Corresponding to Millimeters of 0.1 N *Thiosulfate*

0.1 *N* THIOSULFATE	INVERT SUGAR	0.1 *N* THIOSULFATE	INVERT SUGAR
ml	*mg*	*ml*	*mg*
1	3.2	14	47.3
2	6.4	15	50.8
3	9.7	16	54.3
4	13.0	17	58.0
5	16.4	18	61.8
6	19.8	19	65.5
7	23.2	20	69.4
8	26.5	21	73.3
9	29.9	22	77.2
10	33.4	23	81.2
11	36.8	24	85.2
12	40.3	25	89.2
13	43.8	—	—

Reports have been received from five of the six collaborators, and the results, expressed as per cent of equivalent invert sugar in the original product, are shown in Table 1.

TABLE 1.—*Per cent unfermented reducing substances, expressed as invert sugar*

In blackstrap and refiner's sirup

ANALYST	METHOD I		METHOD II	
	BLACKSTRAP	REFINER'S SIRUP	BLACKSTRAP	REFINER'S SIRUP
1. Sam Byall	4.73	4.28	4.84	4.49
New Orleans, La.	4.84	4.39	5.01	4.30
2. J. K. Dale,	4.54	3.92	4.33	3.92
Terre Haute, Ind.	4.47	3.92	4.31	4.07
3. Carl Erb,	4.54	3.99	4.82	4.00
New York, N. Y.	4.48	3.93	4.86	4.08
4. W. J. Hughes,	4.43	3.99	4.73	4.08
New York, N. Y.	4.49	3.99	4.86	4.00
		3.99	4.73	
5. D. J. Smith,	4.23	3.54	4.79	3.60
Boston, Mass.	4.28	3.57	4.80	3.62
	4.25	3.57		
Averages:				
1	4.79	4.34	4.93	4.40
2	4.51	3.92	4.32	4.00
3	4.51	3.96	4.84	4.04
4	4.46	3.99	4.77	4.04
5	4.25	3.57	4.80	3.61
Grand Averages	4.50	3.96	4.73	4.02

The deviations of the individual averages from the grand averages are shown in Table 2.

As in previous years, each individual collaborator checked his own results very closely. The agreement between the results of the different analysts by both variants of the Java method is of the same order as found previously with the same method. Considering the many manipulations required in carrying out the method, the agreement may be deemed satisfactory.

In both years Analyst 1 obtained in every case the highest results, and Analyst 5 in six cases out of eight the lowest results of all. These tendencies could in the previous series of analyses not be ascribed to differences in the yeast, since the analyses were made upon distillery slop. It is therefore improbable that the yeast used was responsible for the same tendencies observed this year. However, it appears advisable to settle this point definitely in future work, by arranging for the exchange of yeast samples among the collaborators.

TABLE 2.—*Deviations of individual averages from grand averages*

Per cent of invert sugar

ANALYST	METHOD I		METHOD II	
	BLACKSTRAP	REFINER'S SIRUP	BLACKSTRAP	REFINER'S SIRUP
1	+0.29	+0.38	+0.20	+0.38
2	+0.01	−0.04	−0.41	−0.02
3	+0.01	0.00	+0.11	+0.02
4	−0.05	+0.03	+0.04	+0.02
5	−0.25	−0.39	+0.07	−0.41
Average deviations	+0.06	+0.08	+0.08	+0.08
	−0.06	−0.09	−0.08	−0.09
	±0.12	±0.17	±0.16	±0.17
Maximum deviations	0.29	0.39	0.41	0.41

Method II gave on the average slightly higher results than Method I, and both averages and maximum deviations were slightly higher. Since in addition the modified method requires much more time, it offers no particular advantage over the original method.

RECOMMENDATIONS*

On the basis of the results obtained in 1945, and again this year, it is recommended:

(1) That the method of the Java Sugar Experiment Station for the determination of unfermented reducing substances in molasses be adopted as a tentative method.

(2) That the method be subjected to further collaborative study, with arrangements for the exchange of yeast samples among the collaborators.

No reports were made on drying methods, densimetric and refractometric methods, or honey.

REPORT ON CONFECTIONERY

By CHARLES A. WOOD (Food and Drug Administration, Federal Security Agency, New York, N. Y.), *Associate Referee*

In considering the method for lac[1] in candies, it occurred to the writer that removal of sugars, etc., from the impure lac residue thus obtained could be more easily accomplished than by the indicated washing and redrying.

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* For report of Subcommittee D, and action by the Association, see *This Journal*, **31**, 109 (1948).

[1] *This Journal*, **30**, 289 (1946).

It was found that the impure lac residue could be dissolved in a mixture of isoamyl alcohol and benzol and this could be washed free of water soluble matter in a separatory funnel.

Recovery of known amounts of shellac seemed satisfactory, as shown in Table 1.

TABLE 1.—*Recovery of known amounts of lac*

TYPE OF MATERIAL	AMOUNT OF LAC ADDED	AMOUNT OF LAC RECOVERED
	gm	*gm*
200 g sucrose*	.208	0.216
50 g sugar candy†	.533	0.526

* Dissolved in amyl alcohol-benzol mixture with lac.
† Carried entirely through determination.

It appeared this modification was suitable, therefore the details were included in the method for collaborative testing, as follows:

METHOD FOR LAC IN CANDIES

Place 50 g of candy in a 400 ml beaker. Add 50 ml of a mixture of benzol and absolute alcohol (50% by volume) and cover with a watch-glass. Place on the steam bath, heat to boiling, and simmer for a few minutes, stirring occasionally. Decant the liquid into a tared round glass dish of about 100 ml capacity, having a flat bottom about 2¼ in diam. Repeat, using a similar mixture; and finally rinse twice with two 25 ml portions of absolute alcohol, simmering and stirring each rinsing liquid. With moist sugar candy, avoid overheating to prevent pieces from sticking together.

Add each liquid to the glass dish previously placed over the steam to evaporate the alcohol-benzol mixture. Allow to remain on bath until alcohol is just removed, rotating the dish as it goes to dryness in order to spread the extract uniformly over the bottom surface. Avoid baking the shellac on the dish. If fat appears to be present wash with 3–15 ml portions of petroleum benzene, stirring, and warming. Decant thru a rapid filter.

Add 50 ml of a mixture of 25 ml isoamyl alcohol and 25 ml benzol, rinsing any solid matter off and filter back into dish. Heat on steam bath with stirring, cool somewhat, and transfer the soln with suspended matter to a suitable (125 ml) separatory funnel. Rinse the dish with 25 ml water, adding it to the funnel; shake well, and reject the wash water. Repeat washing *two times* (or until washings are colorless) with water, rinsing the dish well around sides with the first portions of the liquid. Finally, filter the soln of the shellac into the tared dish, rinsing the separator and filter with a little absolute alcohol. Evaporate to dryness on the steam bath, rotating the dish on going to dryness to give a uniform film.

If much fat was extracted in original benzol extraction, wash the final shellac residue with 25 ml petroleum ether, warming and stirring. Decant, dry on steam bath and 100° oven, and weigh.

Three samples for collaborative testing were prepared.

Sample A—sugar candy with 0.912% lac added
Sample B—sugar candy with 0.456% lac added plus 8 ml almond oil to simulate conditions in marzipen-type candies
Sample C—commercial glazed chocolate-covered peanuts.

Results are shown in Table 2.

TABLE 2.—*Collaborators' results on lac in candy*

COLLABORATOR	LAC FOUND—PER CENT		
	A	B	C
J. L. Hogan, F&D Adm., N.Y.	0.92	0.47	.08
		0.47	.09
		0.47	
C. A. Wood, F&D Adm., N.Y.	0.94	0.47	0.20
	0.91	0.47	0.12
K. Breen, N.Y. State Lab., Albany	0.73	0.48	0.18
	0.51	0.50	0.15

Results appear fairly good on samples B and C. In the higher range, at times, incomplete recovery is obtained. Possibly, the solubility limit in the isoamyl alcohol-benzol mixture is approached, and this should be further investigated.

It is recommended* that the method be further studied especially with reference to large amounts of lac.

REPORT ON REDUCING SUGARS

By EMMA J. MCDONALD (National Bureau of Standards, Washington 25, D. C.), *Associate Referee*

I. DENSITY AND REFRACTIVE INDICES OF LACTOSE SOLUTIONS

During the past year the Associate Referee has spent considerable time on densimetric and refractive index studies of lactose solutions. This disaccharide, commonly known as milk sugar, for many years has been of commercial importance in the pharmaceutical and in the baby food industries. During the past few years the demand for lactose has greatly increased because of its use in penicillium manufacture. It is expected that the density and refractive index tables which will soon be published will be of use to the investigator working with pure solutions, as well as to the analyst dealing with solutions in which the total solids may be expressed as lactose.

The lactose used in the investigation was prepared by recrystallization of the commercial product from aqueous solution. This was carried out at temperatures where only the lactose hydrate is formed. Lactose hydrate contains one molecule of water of crystallization, or 5 per cent. The water content of the samples used in the investigation was determined from time to time by drying a sample in a vacuum at 120°C. It was found that some decomposition took place, as indicated by a slight discoloring of the sugar. Comparative rates of drying were run at 130, 120, and 85°C. 120°C. was chosen as the optimum temperature, since there appeared to be less decomposition during the loss of water at this temperature. The importance of drying to a constant weight rather than for a given time should be emphasized, since the rates of drying of samples placed in the oven at the same time was not identical.

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* For report of Subcommittee D and action of the Association, see *This Journal*, 31, 61 (1948).

The solubility of lactose determines the range where accurate measurements of solution properties can be obtained. At 20°C. a saturated solution contains 16 per cent of lactose hydrate. We, therefore, carried out density and refractive index measurements with high precision in the range of unsaturation. The values that will be reported for supersaturated solutions, although not obtained with the same precision, are suitable for many purposes.

The density measurements were carried out in a 126 ml flask, whose volume had been accurately determined. All weighings were reduced to vacuum. The air was removed from the solution before making completely to volume. This was accomplished by placing the flask in an environment of reduced pressure and carefully rotating it.

Refractive index measurements were made with a Zeiss immersion refractometer under carefully controlled conditions of temperature, when working with unsaturated solutions at 20° and 25°C. An Abbé instrument was used for more concentrated solutions at 25°C. and for the entire range of concentration at 15°C. These latter measurements were made in order to determine the temperature coefficient. Attempts to determine the refractive indices of supersaturated solutions at higher temperatures failed because of the ease with which the lactose crystalizes. The data on density and refractive indices for each temperature has been subjected to the method of least squares, and equations thus prepared have been used for calculating these physical constants at various percentage concentrations. In the range of unsaturation the disagreement between the observed densities and those calculated by the formulas thus derived has a maximum value of 3 in the 5th decimal place. At higher concentrations, the data of Schmoeger has been analysed by the same method. Since this work was reported only to the 4th place and since the difficulties of preparing the solution are considerable, it is remarkable that the deviation of the observed data from that of the curve represented by the formula is on an average less than .0002 with a maximum of .0007 for a concentration of 50 per cent. The formula expressing the refractive index of an unsaturated solution as a function of the percentage concentration gives values differing from those observed by a maximum of 2 in the 5th decimal place. This is well within the precision of the measurements. For the higher concentrations at 25°C., and over the entire range of concentration at 15°C. where the values are reported only to the 4th place, the maximum deviation is 2 in this decimal place.

II. THE DETERMINATION OF LACTOSE AND LACTOSE-SUCROSE MIXTURES BY THE MUNSON-WALKER GENERAL METHOD

In continuation of his work on dextrose, levulose, invert sugar, and invert sugar-sucrose mixtures which are at present included in the Book of Methods, Dr. Hammond, of the Bureau of Standards, has now about completed a study of the copper equivalents of lactose and lactose-sucrose mixtures by the Munson-Walker method. The copper has been determined electrolytically, and hence this table will be of value to analysts who determine copper directly rather than as the oxide.

III. MICRO METHOD FOR REDUCING SUGARS

The micro method for reducing sugars proposed by Somogyi is now included in the Book of Methods[1] as a tentative method. A copper-carbonate-bicarbonate reagent is employed in this method. In 1945 the author[2] proposed a reagent containing disodium phosphate and sodium hydroxide which had the advantage of stability over a long period of time. Since this would be of great value to the analyst who is required to make an occasional determination it is recommended* that this modification of the method be studied.

No report was made on corn sirup and corn sugar.

REPORT ON COLOR AND TURBIDITY IN SUGAR PRODUCTS

By JOSEPH F. BREWSTER (National Bureau of Standards, Washington 25, D. C.), *Associate Referee*

Certain changes in nomenclature and symbols used in colorimetry and spectrophotometry have been suggested with the view of simplifying the expression of results. The proposed amendment applies to the section of the author's 1946 report[1] headed "Definitions and Symbols." The changes are to be as follows:

Fourth item: For "transmittance" read "transmittancy."
Fifth item: Eliminate and substitute "$A = -\log_{10}T = \log_{10} 1/T =$ absorbancy."
Sixth item: Eliminate and substitute therefor "$a = A/bc = \log_{10} 1/T/bc =$ absorbancy index." It will be noted that a replaces $-\log t$ formerly used.

It is recommended* that these proposed changes be given consideration when the method for the measurement of color in solutions of sugar products is recommended for adoption either as a tentative or official method.

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[1] *Methods of Analysis*, sixth ed., 34.63–34.65 (p. 578–9).
[2] *J. Biol. Chem.*, 160, 61 (1945).
* For report of Subcommittee D and action by the Association, see *This Journal*, 31, 62 (1948).
[1] *This Journal*, 30, 290 (1947).

REPORT ON FLAVORS AND NON-ALCOHOLIC BEVERAGES

By JOHN B. WILSON (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

Only a little time was available this year for laboratory work on the problems attending the analysis of flavors and non-alcoholic beverages which are recommended for study by the Association, but it is hoped that several of the investigations can be undertaken during the coming year.

The Referee made a short study of the application of the modified oil separatory trap to the determination of essential oil in emulsions. The following procedure was used:

DETERMINATION OF ESSENTIAL OIL IN EMULSIONS

APPARATUS

Boiling Flask: Use a 500 ml round bottom flask with ground glass joint 24/40.

Oil Trap: Use the modified oil separatory trap described in *This Journal*, **27**: 201 (1944).

Condenser: Use a Liebig condenser.

DETERMINATION

Place about 200 ml of H_2O in the boiling flask; measure 5–10 ml of the emulsion (believed to contain not over 2 ml of essential oil) in a glass-stoppered graduated cylinder; add to the flask and wash out the cylinder into the flask by adding several 5 ml portions of H_2O to the cylinder and shaking to remove the emulsion from the sides.

Fill the oil trap with H_2O until it overflows, connect with the boiling flask and condenser and boil for one hour. When the boiling time is over, remove the heat source and let stand for several minutes. Now draw off enough water to bring the oily layer within the graduated portion of the trap, let stand for 5 min to complete drainage, and measure the quantity of oil from bottommost to topmost points of the menisci.

To try out the procedure several emulsions were prepared as follows:

PRIMARY EMULSION

Mix until homogeneous 55 ml of cold pressed lemon oil, 40 g of gum arabic, and 170 ml of water in a beaker, using an electric soda fountain mixer. Make up to 250 ml in a graduated cylinder, adding more water if necessary. Now pass the mixture four times thru a homogenizer (a hand homogenizer called Aluminum Cream Maker was used).

Three secondary emulsions were prepared from the primary emulsion (A) by diluting various volumes of the latter to known volumes with water. In the case of B, 50 ml was diluted to 100 ml and passed through the homogenizer, giving a concentration of 11 ml oil per 100 ml. With C and D, 25 ml and 20 ml, respectively, were diluted to 100 ml and mixed by shaking. These therefore contain 5.5 and 4.4 ml of oil per 100 ml. The results obtained by the application of the procedure given above are given in Table 1.

The results in Table 1 indicate that the modified oil separatory trap can be applied to the determination of essential oils in emulsions. It is possible

TABLE 1.—*Determination of lemon oil in emulsions*

SAMPLE DESIGNATION	VOLUME	VOLUME OF OIL			RECOVERY	OIL BY VOLUME	
		PRESENT	FOUND	CORRECTED[1]		PRESENT	FOUND CORRECTED
	ml	*ml*	*ml*	*ml*	*per cent*	*per cent*	*per cent*
A	6	1.32	1.10	1.22	92.4	22.0	20.3
	8	1.76	1.53	1.70	96.6		21.3
	8	1.76	1.55	1.72	97.7		21.5
	7.1	1.54	1.35	1.50	97.4		21.1
	9.1	2.00	1.75	1.94	97.0		21.3
B	5.4	0.60	0.53	0.59	98.3	11.0	10.9
	9.4	1.03	0.92	1.02	99.0		10.7
C	6.2	0.34	0.28	0.31	91.2	5.5	5.0
	9.6	0.53	0.47	0.52	98.1		5.3
D	6.0	0.26	0.25	0.28	107.7	4.4	4.7
	9.6	0.42	0.40	0.42	100.0		4.3
				Ave.	97.8		

[1] The factor 0.9 was used, as is used for lemon oil, in the Official Steam Distillation Method for oils of lemon, orange, or lime in oil base flavors, *Methods of Analysis*, 1945, sec. 25.33.

that a factor other than that used for lemon oil in oil base flavors will be required, since there is a tendency toward low results. One-half hour of boiling was found insufficient in the case of emulsions, although 30 to 45 minutes sufficed when no gum was present (*loc cit*).

In the sixth edition of *Methods of Analysis* extensive changes were made in the method for alcohol by volume in the chapter on Distilled Liquors. The changes in technique have made it necessary for the Referee to review the procedures for determining alcohol in flavoring extracts and to bring the directions in Chapter 25 into harmony with the new procedure for spirits upon which they are based.

Vanilla extracts may contain as much as 200 grams of sugar, dextrose, or glycerin per liter, as provided in the advisory standard issued under the Federal Food and Drugs Act of 1906.

Ginger extract contains solid matter derived from ginger root and the remaining extracts treated in this chapter contain essential oils. None of these various mixtures can be expected to have the same coefficient of expansion as water solution containing the corresponding proportion of alcohol. Since the alcohol content is legally expressed at 15.56°C., the only way in which the correct alcohol content of such samples may be ascertained is to measure the sample at 15.56°C. With this purpose in view, recommendations are now being made to bring the alcohol methods in Chapter 25 in line with those in Chapter 15.

In cases where essential oils must be removed by salting out and washing with petroleum ether, the method must remain tentative for the present. Other methods are being made official since they refer directly to an official method.

RECOMMENDATIONS*

It is recommended:

(1) That the collaborative study of the reflux method for determination of peel oil in citrus fruit juices and the use of the modified oil separation trap be continued.

(2) That collaborative work be continued on the method for determination of beta-ionone where small amounts are present.

(3) That collaborative studies of the Ripper method for determination of aldehydes in spirits as applied to lemon oils and extracts be continued.

(4) That collaborative studies of the methods proposed by the Referee for determination of esters in lemon extract be continued.

(5) That collaborative studies on the Seeker-Kirby Method for determination of esters in lemon and orange oils (Dept. Agr. Bull. 241) be continued.

(6) That collaborative studies of extract methods containing both isopropyl alcohol and acetone be continued.

(7) That collaborative study of the photometric method for determination of vanillin and coumarin be continued.

(8) That work be continued on the determination of glycerol, vanillin, and coumarin in vanilla and imitation vanilla extracts with special reference to the automatic extraction of vanillin and coumarin.

(9) That the study of emulsion flavors be continued.

(10) That studies on maple concentrates and imitations be continued.

(11) That study of the method for determination of diacetyl, published in *This Journal*, **25**, 255, be continued.

(12) That section **25.2** (p. 365) be changed to read as follows:

"**25.2** Alcohol-Official—Proceed as directed in **16.6** or **16.7** but measure the sample used at 15.56°C. in a pycnometer (see pages 192–193) calibrated at that temperature."

(13) That section **25.23** be changed to read as follows and studied collaboratively:

Measure 50 ml of extract at 15.56°C. in a pycnometer (see page 193) calibrated at that temperature, transfer to 200 ml volumetric flask, washing out the pycnometer several times with H_2O, dilute with H_2O to ca 200 ml; allow mixture to stand until oil separates in a clear layer at top, or centrifuge and add H_2O to bring lower meniscus of oil to mark. Note temperature. Pour mixture into dry Erlenmeyer flask containing 5 g of light $MgCO_3$, stopper, shake well, and filter quickly thru large, dry, folded filter. Pipet 100 ml of filtrate, measured at same temp. into a 300–500 ml distillation flask; add 25 ml of H_2O; and distil almost 100 ml into 100 ml pycnometer (see page 192). Adjust to a convenient temperature, complete volume, mix and determine sp. gr. From the table **44.23** obtain per cent alcohol by volume and compute as under **16.6(b)**. beginning "Compute as follows, etc."

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* For report of Subcommittee D and action by the Association, see *This Journal*, **31**, 60 (1948).

(14) That the statement after the colon in line 3 of **25.54** be deleted and the following substituted:

"Fill 50 ml pycnometer (page 193) with sample at 15.56°C., empty into a separatory funnel containing about 10 g of NaCl. Wash out the pycnometer several times with saturated NaCl, using about 100 ml altogether. Extract twice with 50 ml portions of petroleum benzine (b.p. 40–60°). Collect petroleum benzine extract in second separatory funnel and wash with two 25 ml portions of NaCl soln. Combine original NaCl soln with the washings, add a little powdered pumice, and distil into 100 ml pycnometer (page 192). When almost 100 ml has been distilled make up to mark with H_2O at a convenient temperature and determine alcohol from the sp. gr. as directed under **16.6**, using **44.23**."

(15) That **25.64** be changed to read as follows:

"**25.64** Alcohol-Official—Proceed as directed in **16.6(b)**."

No reports were made on beta-ionone; lemon oils and extracts; organic solvents in flavors; glycerol, vanillin, and coumarin in vanilla and imitation vanilla; emulsion flavors; maple flavor concentrate and imitations; or diacetyl. See Referee report for essential oil in emulsions.

REPORT ON WATERS, BRINE, AND SALT

By A. E. Mix (Food and Drug Administration, Federal Security Agency, Washington, 25, D. C.), *Referee*

Some time ago a short reference appeared in one of the chemical journals suggesting the use of dye index No. 1073 for the detection of small amounts of boron in salt.

The Referee used dye No. 1073 and found that the results were of no value. After several trials it was found that index No. 1073 is used for the sulfonated as well as for the unsulfonated dye.

The unsulfonated dye Violet No. 2, when mixed with concentrated sulphuric acid, produces a dark green color.

It was found that when .01 gram of the dye was dissolved in about 25 ml of concentrated sulfuric acid, and portions from 1 ml to 5 or more ml of this mixture each made up to a final volume of 100 ml with sulfuric acid, the resulting solution had a definite green color.

The first trial was made with a spoonful of sodium chloride A.C.S., placed on a watch-glass with a drop of normal sodium hydroxide in the center of the salt; this was dried in a hot air oven for one hour, cooled, and then 10 drops of the dye solution was dropped on top of the salt, while at the same time another lot of salt was treated with a few fine granules of sodium borate, sodium hydroxide, and the dye.

The salt containing the sodium borate showed a definite blue color, while the other salt sample showed a green color.

Some water solution containing .02 milligram of sodium borate treated with a drop of 1 *N* sodium hydroxide, dried and treated with the dye, showed a definite blue color.

If nitrates interfere, they are removed with concentrated sulfuric acid and hydrazine sulfate. Fluorides retard the action of the dye for about 5 minutes. More work must be done in order to make this a quantitative method.

RECOMMENDATIONS*

It is recommended:

(1) That the method for the determination of boron in water and salt be further studied.

(2) That the determination of fluorine in salt work be completed next year.

No report was given on boron in water or fluorine in salt.

REPORT ON FERTILIZERS

By F. W. QUACKENBUSH (Purdue University Agricultural Experiment Station, Lafayette, Ind.), *Referee*

During the year a new Associate Referee on Sampling was appointed and work was initiated to study sampling methods and containers. Reports were submitted by referees on sampling, phosphoric acid, moisture, nitrogen, and potash. Some others have carried on work, but they felt that further studies should be made before making a report. It is hoped that others will make good progress on their respective assignments and give this group the benefit of their findings next year.

RECOMMENDATIONS†

It is recommended—

(1) That the study of sampling equipment and sampling methods be continued. That line 6 of section 2.1 of *Methods of Analysis A.O.A.C.* (1945) under "Directions for Sampling" be changed to read as follows: "If less than 100 bags, sample not less than 20 bags." Other recommendations of the Associate Referee for adding directions on sampling bulk and small package fertilizers are approved (official first action).

(2) That work on methods for phosphoric acid be continued with emphasis on **(a)** the ammonium citrate method and its applicability to basic slag; **(b)** evaluation of sintered, fused, and calcined phosphates as fertilizers; **(c)** aging of molybdate solutions.

(3) That the recommendations of the Associate Referee on P_2O_5 be adopted, official first action.

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* For report of Subcommittee D and action by the Association, see *This Journal*, 31, 62 (1948).

† For report of Subcommittee A and action by the Association, see *This Journal*, 31, 41 (1948).

(4) That attempts to work out improved methods for moisture be continued.

(5) That work on methods for nitrogen be continued with special emphasis on fertilizers which contain ammonium nitrate.

(6) That work on methods for manganese and magnesium be continued.

(7) That work on problems of acid- and base-forming quality of fertilizers be continued.

(8) That work on potash be continued.

(9) That 95 per cent ethyl alcohol be adopted instead of 80 per cent alcohol in the determination of potash:

(a) That section **2.40(d)** p. 31, *Methods of Analysis* (1945) be changed to read: (d) *Acid-Alcohol.*—Mix 200 ml. of 95% ethyl alcohol with 20 ml. of concentrated HCl and cool to room temperature (official, first action).

(b) That, in section **2.42(a)**, p. 32, *Methods of Analysis* (1945), all references to 80% alcohol be changed to 95% ethyl alcohol (official, first action).

(10) That work on analysis for sulfur be continued.

(11) That the study of methods for copper and zinc be continued.

(12) That work on methods of analysis for boron be continued.

(13) That editorial correction in section **2.10** (b) of "323.81" to "324.03" line 1, and of "32.38" to "32.40," line 2, be made.

REPORT ON SAMPLING FERTILIZERS*

By H. R. ALLEN (Kentucky Agricultural Experiment Station, Lexington, Ky.) *Associate Referee*

Ross, Hardesty, and Rader[1] reported results of a questionnaire on sampling fertilizers. The most widely used triers were the slotted single-tube trier and the slotted double-tube trier, usually known as the Indiana sampler. These investigators cited published papers on this subject to November 1940. These papers represented work done between the years 1916 and 1927.

Use of the Indiana trier theoretically should provide the most representative sample, since nothing can enter the trier while it is being inserted or removed from the bag. This sampler is cumbersome to use, however, and is harder to clean than the single-tube triers. It is generally conceded that all the triers should have one end closed and pointed. It is probable that a bag should not be sampled in the vertical position with a single-tube trier, since a large portion of the sample might be obtained from the upper portion of the bag.

This report gives analyses of samples taken from the same bags with these two types of triers. The report also compares the moisture content of samples sealed in glass when taken and of samples placed in paper envelopes at time of sampling.

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* This investigation, made in connection with a project of the Kentucky Agricultural Experiment Station, is published by permission of the Director.

[1] Ross, W. H., Hardesty, J. O., Rader, L. F., Jr., *This Journal*, 24, 499–506 (1941).

Haigh[2] compared analyses of samples taken with the single-tube and double-tube triers, but the fertilizers were lower in grade than those sampled here and the single-tube trier took a smaller core.

EXPERIMENTAL WORK

Comparison of Triers by Analysis of Samples.—Both triers had one end closed and pointed and the other end open. The single-tube trier had a single slot 24 inches long and ½ inch wide and the tube had an inside diameter of ¾ inch. The effective opening of the Indiana or double-tube trier was 24 inches, separated by ribs into 3 slots each 8 inches long. The width of the slot was ½ inch and the inside diameter of the tube was ⅝ inch.

Samples were taken from 3 shipments of fertilizer with the 2 triers. Each shipment was in paper bags and the bags were stacked in the horizontal position. Cores were taken from 6 bags in the first shipment and from 10 bags in the other shipments. For the composite samples, one core was taken from each bag with the single-tube trier and immediately a core was taken from the same bag, and in the same place, with the double-tube trier. Some of the cores were taken parallel to sides of the bags and some were taken diagonally through the bags, but the same procedure was followed with each trier. Cores taken with the single-tube trier constituted one sample from each shipment and those taken with the double-tube trier constituted another sample. The composite samples were taken with the bags in the horizontal position. The single-tube trier was inserted in the bag with the slot down, then the trier was turned over, opened to admit sample, and closed before withdrawal. The samples were sealed in glass jars.

Next, for each shipment, with the bag in a horizontal position, a core was taken with the single-tube trier from 1 bag, then a core with the double-tube trier in the same place from this bag. This was repeated until 3 cores were taken from the same bag with each trier, these cores constituting 2 samples. Next, this procedure was repeated with the bag in the vertical position.

The entire amount of each sample was ground to pass a sieve with 1 mm openings and the samples were analyzed for moisture, nitrogen, total phosphoric acid, and potash. The results, as reported in Table 1, are averages of 2 closely agreeing duplicate determinations.

Comparison of Moisture in Samples Placed in Glass and Paper Containers when Sampled.—The moisture study was thought advisable, since some control agencies send samples to the laboratory in paper containers. Two samples were taken from each of 5 stocks of fertilizer, 10 bags being

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[2] Haigh, L. D., *ibid.*, 4, 597–599 (1921).

TABLE 1.—*Analyses of samples taken with single-tube and double-tube triers*

Results in per cent*

SAMPLE NUMBER†	MOISTURE	TOTAL NITROGEN	TOTAL PHOSPHORIC ACID	POTASH
No. 1 Samples, 6-8-6				
1 A	7.01	6.07	8.55	6.90
1 B	6.97	6.02	8.55	6.81
1 C	6.77	6.20	8.57	6.91
1 D	6.57	6.28	8.65	6.87
1 E	6.67	6.31	8.35	6.96
1 F	6.50	6.27	8.55	7.00
No. 2 Samples, 4-12-4				
2 A	5.61	4.08	12.35	4.01
2 B	5.58	4.10	12.35	4.03
2 C	5.03	4.10	12.20	3.84
2 D	5.07	4.11	12.25	3.84
2 E	5.03	4.08	12.25	3.92
2 F	4.97	4.08	12.20	3.94
No. 3 Samples, 6-8-6				
3 A	8.10	5.60	10.70	5.68
3 B	8.12	5.62	10.67	5.74
3 C	7.77	5.74	10.50	5.81
3 D	7.76	5.77	10.50	5.80
3 E	7.50	5.77	10.65	5.77
3 F	7.03	5.75	10.65	5.69

* Results are averages of 2 analyses, except some of the moisture figures

† A—Composite sample, with single-tube trier, horizontal position
B—Same sample, with double-tube trier, horizontal position
C—1 bag horizontal position, single tube trier
D—Same bag horizontal position, double-tube trier
E—Same bag vertical position, single-tube trier
F—Same bag vertical position, double-tube trier.

Composite samples were from 6 bags for No. 1 samples and from 10 bags each for No. 2 and No. 3 samples.

sampled in each case. Two cores were taken from each bag sampled, from approximately the same place in the bag. One core from each bag constituted a sample which was sealed in glass when taken. The other core from each bag was placed in an envelope[3] made of heavy kraft paper, open at one end. The envelope is closed by folding the flap as creased in 3 folds, and the flap is inserted in pocket on side of envelope. The samples were stored in the inspector's car until delivered to the laboratory, at which time the samples in paper were transferred to glass jars which were sealed. The samples were in paper containers from 4 to 6 days.

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[3] Spear Safety Envelope, manufactured by Heinrick Envelope Co., Minneapolis, Minn.

Each sample was well mixed and about 150 grams were ground in a mortar to pass a sieve with 1 mm openings. The ground part of the sample was kept in a covered container until the grinding was finished. Tops of laboratory bottles holding the samples were sealed with adhesive tape. Duplicate moisture determinations were made, the samples being dried at 98°–100°C. for 3 hours. The results reported in Table 2 are averages of duplicate determinations. The duplicates with one exception agreed within 0.1 per cent moisture.

TABLE 2.—*Per cent moisture of samples placed in glass jars and in paper envelopes when sampled**

SAMPLE NUMBER	SAMPLES IN GLASS	SAMPLES IN PAPER ENVELOPES
4 A and 4 B†	6.53	5.22
5 A and 5 B	6.92	5.48
6 A and 6 B	4.40	3.37
7 A and 7 B	7.82	6.64
8 A and 8 B	9.17	7.58

* Composite samples from 10 bags in each case. Results are averages of 2 analyses.

† A samples placed in glass jars and B samples placed in paper envelopes when taken. B samples transferred to glass jars after 4 to 6 days.

DISCUSSION OF RESULTS

Comparison of Triers.—Comparison analyses made with 2 types of triers were in close agreement, both for the composite samples and for samples representing individual bags. This might indicate very homogeneous mixtures but each stock of fertilizer sampled was not entirely homogeneous. This is shown by the nitrogen results from the individual bags representing shipments 1 and 3 and by the potash results from the individual bags representing shipment 2.

Probably this work should be supplemented with a comparison of different triers on mixtures that have a tendency to segregate and on samples taken before and after segregation.

Comparison of Moisture.—Marked differences were found in moisture in samples sealed in glass and in those placed in paper containers at time of sampling. The latter group contained from 1 to 1.5 per cent less moisture. It is probable that a considerable part of this loss was due to absorption of moisture by the paper containers. These results indicate that samples should be placed in glass or other non-absorptive containers when taken. Decrease of 1.5 per cent moisture for a guarantee of 6 in any nutrient theoretically means an increase of .09 in percentage results and for a guarantee of 20, the increase is 0.30 per cent.

The writer found even greater differences and in the same direction in comparative samples taken in October and November, 1940. Moisture was compared on samples placed in glass, cellophane and paper. The moisture decrease was less in cellophane than in paper.

SUMMARY

Analyses of samples taken with single-core and double-core triers were in good agreement. Samples from individual bags sampled in horizontal and vertical positions were uniform in analysis.

Samples kept in paper containers for 4 to 6 days after sampling contained from 1 to 1.5 per cent less moisture than samples from same bags which were placed in glass jars and sealed when sampled.

ACKNOWLEDGMENT

The writer is indebted to Miss Lelah Gault for making the phosphoric acid analyses, and to Inspectors Robert Mathews, N. J. Howard, and Neville Hulette for taking the samples.

RECOMMENDATIONS*

It is recommended that—

(1) That study of sampling fertilizers be continued.

(2) That an investigation be made of sampling fertilizers in bulk.

(3) That sec. **2.1**, line 6, be changed to read as follows: "If less than 200 bags, sample not less than 20 bags."

(4) That the following be added to sec. **2.1**, line 7, before the final sentence "In sampling a bulk lot of fertilizer, draw not less than 20 cores from different regions of the lot. In sampling fertilizer packaged in small containers (10 pounds or less) a single package may constitute a sample for the lot."

REPORT ON PHOSPHORIC ACID

A. EFFECT OF CONTINUOUS AGITATION DURING CITRATE DIGESTION ON DETERMINATION OF CITRATE-INSOLUBLE P_2O_5

B. EFFECT OF SULFATE ON DETERMINATION OF P_2O_5 BY THE VOLUMETRIC METHOD

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A. EFFECT OF CONTINUOUS AGITATION DURING CITRATE DIGESTION ON DETERMINATION OF CITRATE-INSOLUBLE P_2O_5

At the 1943 meeting of this Association, MacIntire, Marshall, and

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* For report of Subcommittee A and action by the Association, see *This Journal*, **31**, 41 (1948).

TABLE 1.—*Summary of published data relating to effect of continuous agitation during citrate digestion on results for citrate-insoluble P_2O_5*

SAMPLE	MATERIAL	TOTAL P_2O_5	CITRATE-INSOLUBLE P_2O_5[a] PERIODIC AGITATION[b]	CITRATE-INSOLUBLE P_2O_5[a] CONTINUOUS AGITATION[c]	CITRATE-INSOLUBLE P_2O_5[a] DIFFERENCE
		per cent	*per cent*	*per cent*	*per cent*
	Results given by Jacob, Rader, and Tremearne (9)				
1374	Calcined phosphate[d]	35.17	3.91	3.79	0.12
1351	Calcined phosphate[d]	36.58	11.80	11.79	0.01
1395	Rhenania phosphate	30.42	3.70	0.43	3.27
1396	Rhenania phosphate	25.66	2.18	1.84	0.34
1110	Non-acid phosphate	26.50	11.42	11.00	0.42
1164	Basic slag	18.45	3.74	3.47	0.27
1107	Basic slag	23.36	4.55	3.38	1.17
	Results given by MacIntire, Marshall, and Meyer (11)				
A-1	Superphosphate	20.44	0.03	0.01	0.02
A-2	Superphosphate	20.13	0.02	0.02	0.00
A-4	Superphosphate	18.75	0.06	0.03	0.03
3	Triple superphosphate	47.50	2.99	2.95	0.04
A-10	Ammoniated superphosphate	20.63	1.97	1.72	0.25
A-15	Ammoniated superphosphate	19.37	2.75	2.60	0.15
A-14	Ammoniated superphosphate	19.28	2.88	2.80	0.08
A-11	Ammoniated superphosphate	20.00	2.45	2.25	0.20
A-9	Ammoniated superphosphate	18.79	6.35	6.15	0.20
A-13	Ammoniated superphosphate	19.25	6.93	6.85	0.08
3	Ammoniated superphosphate	41.38	2.40	2.40	0.00
4	Ammoniated superphosphate	41.19	4.58	4.42	0.16
A-5	Limed superphosphate	17.12	4.18	3.68	0.50
1	Monocalcium phosphate	56.32	0.00	0.00	0.00
2	Dicalcium phosphate, crude	51.75	2.60	1.98	0.62
4	Calcined phosphate[d]	37.06	7.54	4.98	2.56
10	Calcined phosphate[d]	36.88	3.65	3.48	0.17
11	Calcined phosphate,[d] reverted	36.63	19.70	19.63	0.07
12	Basic slag, imported	17.13	3.01	2.80	0.21
B-11	Basic slag, fluospar	10.75	9.25	9.25	0.00
13	Calcium metaphosphate	61.88	0.08	0.06	0.02
14	Fused phosphate[e]	26.79	3.75	2.95	0.80
B-12	Bone ash	40.75	35.13	35.00	0.13
—	Phosphate rock[f]	35.31	32.19	31.88	0.31
5	Mixed fertilizer, 6-8-4	21.96	13.35	13.28	0.07
6	Mixed fertilizer, 6-9-4	9.88	0.79	0.75	0.04
4	Mixed fertilizer, 3-10-5	14.88	4.68	4.62	0.06
2	Mixed fertilizer, 5-10-5	15.88	4.88	4.73	0.15
1	Mixed fertilizer, 6-12-6	18.07	3.45	3.40	0.05

[a] In all the determinations, 1-gram samples were digested with 100 ml. of neutral ammonium citrate solution for 1 hour at 65°C. in the presence of filter paper.

[b] Official method; manual shaking at 5-minute intervals.

[c] End-over-end rotation at 12 r.p.m. by Jacob *et al.* and 20 r.p.m. by MacIntire *et al.*

[d] Calcined alpha phosphate.

[e] Fused alpha phosphate.

[f] Florida pebble, National Bureau of Standards sample No. 120.

Meyer (11) pointed out the disadvantages of the official method for citrate-insoluble P_2O_5 (*Methods of Analysis*, 1, pp. 23–24), with emphasis on the amount of the analyst's time required for the periodic shaking of the flasks and on the disagreeable features of this operation, as well as the difficulty, if not impossibility, of maintaining strict uniformity of temperature in all portions of the bath and throughout the digestion period. In the same paper a description is given of an electrically-heated cabinet provided with means for continuous end-over-end rotation of the flasks at 20 r.p.m. and for the maintenance of their contents at a constant temperature of 65°C. It is stated that better reproducibility of values for citrate-insoluble P_2O_5 are obtained with the use of this apparatus than with the techniques of the official method.

In an earlier paper[1] Jacob, Rader, and Tremearne (9) report values for citrate-insoluble P_2O_5 in several types of water-insoluble phosphates—calcined phosphate (calcined alpha phosphate), Rhenania phosphate, non-acid phosphate, and basic slag—as determined, respectively, by the official method (shaking at 5-minute intervals) and by continuous end-over-end rotation at 12 r.p.m. in a thermostat. These results, together with those obtained by MacIntire, Marshall, and Meyer (11) on a much wider variety of materials, are summarized in Table 1. It will be noted that continuous agitation gave values identical with or lower than those obtained by the official method. In general, the differences are small and do not exceed 0.5 per cent P_2O_5 except in the case of certain samples of Rhenania phosphate, calcined phosphate, and fused phosphate (fused alpha phosphate).

In view of the fact that the MacIntire-Marshall-Meyer apparatus has been placed on the market and is now used by a number of fertilizer laboratories (including several engaged in State fertilizer-control work), although continuous agitation during the citrate digestion has not yet been adopted officially, the Referee on Fertilizers in his report to the 1946 meeting of the Association (6) recommended "that a study be made of the use of mechanical shakers in the method for citrate-insoluble phosphoric acid to ascertain whether modifications should be made in the method for water-soluble and citrate-insoluble phosphoric acid to permit the use of mechanical shakers." Pursuant to this recommendation a collaborative investigation of the subject was carried out and the results are presented herein.

SAMPLES

The samples submitted to the collaborators are listed in Table 2, which also shows the total P_2O_5 content of the materials. Samples 1, 2, 3, 4, 13, and 14 were ground to pass a 40-mesh sieve and the others to pass a 100-mesh sieve.

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[1] Presented at the 1935 meeting of this Association.

TABLE 2.—*Samples for collaborative study of effect of continuous agitation during citrate digestion results for insoluble P_2O_5*

SAMPLE	MATERIAL	TOTAL P_2O_5[a]		
		COLLABORATOR 4	COLLABORATOR 7	AVERAGE
		per cent	*per cent*	*per cent*
1	Ammoniated superphosphate, 3.45% *N*	20.34	20.02	20.18
2	Ammoniated superphosphate, 4.87% *N*	19.69	19.52	19.60
3	Mixed fertilizer, 4-12-6	12.45	12.45	12.45
4	Mixed fertilizer, 4-12-6	12.30	12.32	12.31
5	Dicalcium phosphate	48.60	48.32	48.46
6	Fused alpha phosphate[b]	27.69	27.68	27.68
7	Sintered alpha phosphate[c]	20.47	20.54	20.50
8	Calcined alpha phosphate[d]	37.23[e]	37.25	37.24
9	Phosphate rock-magnesium silicate glass[f]	20.27[g]	20.60	20.43
10	Basic slag, low analysis	8.21[h]	8.42	8.31
11	Basic slag, high analysis	16.12	16.15	16.13
12	Phosphate rock, Florida pebble	30.92	30.70	30.81
13	Superphosphate, Florida pebble	21.14	20.78	20.96
14	Superphosphate, Tennessee brown rock	—	20.44	20.44

[a] By acid digestion of the sample.
[b] 0.34% F.
[c] 0.05% F.
[d] 0.09% F.
[e] 37.30% by NaOH fusion plus acid digestion of the sample.
[f] 1.70% F.
[g] 20.47% by NaOH fusion plus acid digestion of the sample.
[h] 8.33% by NaOH fusion plus acid digestion of the sample.

Superphosphates.—The samples (Nos. 13 and 14) are well-cured run-of-pile materials manufactured from Florida pebble and Tennessee brown-rock phosphates, respectively. Sample 14 was submitted to only one collaborator.

Ammoniated Superphosphates.—The samples (Nos. 1 and 2) were prepared in the laboratory by treating Florida pebble superphosphate (Sample 13) with anhydrous ammonia to yield products containing 3.45 and 4.87 per cent nitrogen, respectively.

Mixed Fertilizers.—The phosphate component in Samples 3 and 4 was supplied by ammoniated superphosphates No. 1 and 2, respectively. The other constituents of the mixed fertilizers are ammonium sulfate, potassium chloride, dolomite, Hyperhumus, and sand (make-weight filler).

Dicalcium Phosphate.—This material (Sample 5) is a high-grade product manufactured by the Tennessee Valley Authority from electric-furnace phosphoric acid.

Alpha Phosphates[2].—The fused alpha phosphate (Sample 6) and the sintered alpha phosphate (Sample 7) are commercial-scale products made, respectively, from Tennessee brown-rock and Florida land-pebble phosphates by the Tennessee Valley Authority, Godwin (near Columbia), Tennessee, and by the Coronet Phosphate Company, Plant City, Florida, in 1946. The calcined alpha phosphate (Sample 8) was prepared about 1935 from Tennessee brown-rock phosphate on a pilot-plant scale.

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[2] The term *alpha phosphate* (10) is used to denote the products obtained by heating natural phosphates, usually phosphate rock, at temperatures above 1300°C. in the presence of silica and water vapor for the purpose of volatilizing the fluorine and converting the P_2O_5 into plant-available forms (4, 7, 12, 13, 17). It does not include the product (all glass) obtained by fusing a mixture of phosphate rock and magnesium silicate or low-fluorine phosphate of the Rhenania type. The term now embraces three types of materials—fused, sintered, and calcined—all of which are frequently called "defluorinated phosphate rock" or "defluorinated phosphate." While fused alpha phosphate is currently called "fused tricalcium phosphate," it

Phosphate Rock-Magnesium Silicate Glass.—The results of experiments on production of available phosphate by fusing phosphate rock with magnesium silicate have been described by Walthall and Bridger (20), and the composition, properties, "solubility," and fertilizing value of several experimental and commercial products of this kind are discussed in a recent paper by Hill *et al.* (8). Large-scale manufacture of the material was achieved in 1946 by the Permanente Metals Corporation, Permanente, California, by fusing a proportioned mixture of Idaho phosphate rock and serpentine in electric furnaces at approximately 1500°C. and quenching the tapped melt to a glass in a violent spray of water. The product is sold under the name of Thermo-Phos. The material (Sample 9) used in the present study is from Permanente's production in late 1946.

Basic Slags.—Sample 10 is a low-analysis material produced in the Birmingham, Alabama, district, while Sample 11 is a high-analysis material imported from Europe prior to World War II.

Phosphate Rock.—The sample (No. 12) is commercial material from the Florida land-pebble district.

COLLABORATORS' DIRECTIONS FOR ANALYSIS

1. Determine citrate-insoluble P_2O_5 as directed in *Methods of Analysis, A.O.A.C.*, 1945, pp. 23–24, sec. **2.15** and **2.16(a)**, in the case of the samples (Nos. 1, 2, 3, 4, 13, and 14) that contain water-soluble P_2O_5. In the case of the samples (Nos. 5 to 12) that contain little or no water-soluble P_2O_5 determine citrate-insoluble P_2O_5 as directed in sec. **2.15** and **2.16(b)**.

2. Repeat the determinations of citrate-insoluble P_2O_5 as follows: Proceed as directed in sec. **2.15** and **2.16(a)** or sec. **2.15** and **2.16(b)**, depending on the nature of the sample, through the point where the flask is first shaken vigorously to reduce the filter paper to a pulp. Next, place the flask in a continuous shaking or stirring device enclosed in a water or air bath provided with means for maintaining the contents of the flask at exactly 65°C., and agitate for exactly 1 hour from the time the sample was introduced into the flask. Then proceed with the determination as before.

3. As it is desired to subject the results for citrate-insoluble P_2O_5 to statistical analysis, it is requested that with each sample and with each method of agitating the contents of the flask the determinations be made in triplicate, *i.e.*, one determination on each of three separate 1-gram portions of the sample for each set of conditions. It is necessary that the result of each determination be reported individually.

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has also been designated as "fused rock phosphate," "fused phosphate rock," and "fused phosphate." Sintered alpha phosphate is marketed under the name of "Coronet defluorinated phosphate," but it has also been called "calcined phosphate." Calcined alpha phosphate has not been produced on a commercial scale; it has generally been called "calcined phosphate." The composition, properties, and "solubility" of the alpha phosphates are discussed in the report of the Associate Referee on Phosphoric Acid presented at the 1946 meeting of this Association (10).

NOTES

(a) Most of the collaborators will have access to the constant temperature, continuous agitation (end-over-end rotation) device described by MacIntire, Marshall, and Meyer (11), and it is recommended that this apparatus be used. Also, it is very desirable to have results by other continuous agitation devices provided with means for maintaining the contents of the flask at a constant temperature of 65°C. If you have such devices at your disposal or can readily assemble them, please make additional determinations with their aid. In case devices other than the MacIntire-Marshall-Meyer apparatus are used, please indicate the type and the speed of rotation or stirring.

(b) Determination of total P_2O_5 is desirable but is not required. If such determination is made, prepare the solution as directed in *Methods of Analysis, A.O.A.C.*, 1945, pp. 21–22, sec. **2.8(a)** or **2.8(b)** and proceed as directed in sec. **2.10** and **2.12(a)**, pp. 22–23.

(c) It is desirable that you determine citrate-insoluble P_2O_5 in all the samples. If this is not feasible, however, please study at least those samples marked with an asterisk in the accompanying list.

(d) Your comments and observations concerning this investigation are requested, especially regarding the advantages or disadvantages of mechanical continuous agitation as compared with manual shaking at 5-minute intervals.

COLLABORATORS

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2. Blackwell, A. T., The Davison Chem. Corp., Baltimore, Md.
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4. Elmore, K. L., Tenn. Valley Authority, Wilson Dam, Ala.
5. Hardin, L. J., and Johnson, H. S., Jr., Tenn. Valley Authority, Knoxville, Tenn.
6. Lawton, J. K., Fla. Agr. Dept., Tallahassee, Fla.
7. Pinckney, R. M., and Rader, L. F., Jr., Bur. Plant Ind., Soils, and Agr. Eng., Beltsville, Md.
8. Villa-Mayo, R., and Segura, P., Puerto Rico Dept. Agr. and Com., San Juan, P. R.
9. North Carolina Department of Agriculture, Raleigh, N. C.

PROCEDURES

With one exception, all the collaborators compared the official method (shaking at 5-minute intervals) with continuous end-over-end rotation in the MacIntire-Marshall-Meyer apparatus at 20 r.p.m. Collaborator 2 compared the official method and continuous stirring at approximately 200 r.p.m. In addition to the official and the MacIntire-Marshall-Meyer methods, Collaborators 3 and 7 made determinations with the aid of other continuous agitation devices enclosed in thermostatically controlled, electrically heated cabinets, as follows:

(1) Ross-Kershaw shaker (18) with movement in the horizontal plane at a rate of approximately 345 oscillations per minute, using tightly stoppered flasks.

(2) Continuous stirring at about 350 r.p.m., using loosely stoppered flasks.

In the determinations by continuous agitation, Collaborators 1–5 and 7 preheated the citrate solution in a water bath, while Collaborators 6, 8, and 9 preheated the solution in the cabinet itself.

TABLE 3.—*Effect of continuous end-over-end rotation during citrate digestion on results for citrate-insoluble P_2O_5*

COLLABORATOR	CITRATE-INSOLUBLE P_2O_5 BY— METHOD I[a]		METHOD II[b]		DIFFERENCE IN AVERAGE RESULTS[c]
	RANGE	AVERAGE	RANGE	AVERAGE	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
	Sample 1. Ammoniated Superphosphate				
1	1.60–1.65	1.62	1.55–1.60	1.58	0.04
3	0.81–0.93	0.87	0.59–0.64	0.62	0.25*
4	1.79–1.82	1.81	1.52–1.56	1.54	0.27*
5	1.35–1.45	1.42	1.15–1.25	1.18	0.24*
6	1.55–1.65	1.60	1.70–1.85	1.76	−0.16*
7	1.70–1.79	1.75	1.60–1.73	1.67	0.08
8	1.38–1.50	1.43	1.38–1.48	1.41	0.02
9	2.12–2.25	2.18	1.95–2.27	2.08	0.10
Average	—	1.59	—	1.48	0.11*
	Sample 2. Ammoniated Superphosphate				
1	3.25–3.35	3.30	2.90–3.00	2.97	0.33*
3	1.89–2.10	2.03	1.54–1.63	1.57	0.46*
4	1.92–2.52	2.22	1.74–1.83	1.78	0.44*
5	1.97–2.00	1.98	1.75–1.80	1.77	0.21
6	2.20–2.40	2.30	2.25–2.55	2.43	−0.13
7	2.45–2.78	2.65	2.83–3.19	3.03	−0.38*
9	3.30–3.50	3.42	3.07–3.22	3.13	0.29*
Average	—	2.56	—	2.38	0.18*
	Sample 3. Mixed Fertilizer				
1	0.15–0.20	0.18	0.15–0.20	0.17	0.01
3	0.19–0.20	0.19	0.18–0.20	0.19	0.00
4	0.15–0.29	0.20	0.08–0.08	0.08	0.12*
5	0.15–0.18	0.17	0.12–0.13	0.13	0.04
6	0.20–0.35	0.28	0.25–0.30	0.28	0.00
7	0.12–0.13	0.13	0.21–0.35	0.28	−0.15*
8	0.27–0.30	0.28	0.24–0.29	0.27	0.01
9	0.47–0.50	0.49	0.40–0.47	0.43	0.06
Average	—	0.24	—	0.23	0.01
	Sample 4. Mixed Fertilizer				
1	0.25–0.30	0.28	0.20–0.20	0.20	0.08*
3	0.28–0.31	0.29	0.18–0.21	0.20	0.09*
4	0.15–0.31	0.21	0.09–0.14	0.11	0.10*
5	0.20–0.23	0.21	0.18–0.19	0.18	0.03
6	0.30–0.35	0.33	0.25–0.30	0.27	0.06*
7	0.25–0.33	0.29	0.29–0.37	0.34	−0.05
9	0.60–0.65	0.62	0.47–0.55	0.51	0.11*
Average	—	0.32	—	0.26	0.06*

[a] Official; manual shaking at 5-minute intervals.

[b] Continuous end-over-end rotation in MacIntire-Marshall-Meyer apparatus (11).

[c] The minus sign denotes that the result by method II is higher than that by method I. The asterisk denotes that the difference is statistically significant at the 5% level.

TABLE 3.—(*continued*)

COLLABORATOR	CITRATE-INSOLUBLE P_2O_5 BY—				DIFFERENCE IN AVERAGE RESULTS[c]
	METHOD I[a]		METHOD II[b]		
	RANGE	AVERAGE	RANGE	AVERAGE	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
	Sample 5. Dicalcium Phosphate				
1	0.60–0.60	0.60	0.60–0.60	0.60	0.00
3	0.62–0.63	0.63	0.61–0.62	0.62	0.01
4	0.54–0.55	0.55	0.55–0.57	0.55	0.00
5	0.41–0.42	0.42	0.40–0.42	0.41	0.01
6	0.65–0.85	0.75	0.70–0.75	0.73	0.02
7	0.56–0.59	0.58	0.65–0.83	0.73	−0.15*
8	0.52–0.67	0.61	0.66–0.69	0.67	−0.06
9	0.72–0.75	0.74	0.70–0.75	0.72	0.02
Average	—	0.61	—	0.63	−0.02
	Sample 6. Fused Alpha Phosphate				
1	5.30–5.35	5.32	5.25–5.35	5.30	0.02
3	5.18–5.20	5.19	4.73–4.82	4.77	0.42*
4	4.79–4.94	4.87	4.38–4.44	4.41	0.46*
5	5.42–5.50	5.45	4.75–4.80	4.77	0.68*
6	5.05–5.30	5.18	5.10–5.15	5.12	0.06
7	5.28–5.57	5.44	4.96–5.18	5.09	0.35*
9	6.15–6.35	6.22	4.75–4.87	4.82	1.40*
Average	—	5.38	—	4.90	0.48*
	Sample 7. Sintered Alpha Phosphate				
1	1.55–1.60	1.57	1.55–1.55	1.55	0.02
3	1.59–1.61	1.60	1.53–1.54	1.53	0.07
4	1.46–1.59	1.50	1.34–1.46	1.40	0.10*
5	1.60–1.65	1.62	1.55–1.60	1.58	0.04
6	1.55–1.70	1.62	1.55–1.65	1.62	0.00
7	1.68.1.72	1.70	1.65–1.67	1.66	0.04
8	1.59–1.61	1.60	1.58–1.73	1.64	−0.04
9	1.62–1.72	1.67	1.47–1.50	1.49	0.18*
Average	—	1.61	—	1.56	0.05*
	Sample 8. Calcined Alpha Phosphate				
1	3.25–3.35	3.30	3.25–3.35	3.30	0.00
3	3.34–3.35	3.34	3.09–3.10	3.10	0.24*
4	2.80–2.86	2.82	2.68–2.76	2.72	0.10*
5	2.62–2.70	2.67	2.62–2.75	2.69	−0.02
6	3.05–3.20	3.12	3.10–3.15	3.15	−0.03
7	3.38–3.54	3.43	3.42–3.56	3.50	−0.07
9	3.67–3.77	3.72	3.20–3.35	3.25	0.47*
Average	—	3.20	—	3.10	0.10*

TABLE 3.—(*continued*)

COLLABORATOR	CITRATE-INSOLUBLE P_2O_5 BY—				DIFFERENCE IN AVERAGE RESULTS[c]
	METHOD I[a]		METHOD II[b]		
	RANGE	AVERAGE	RANGE	AVERAGE	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
	Sample 9. Phosphate Rock-Magnesium Silicate Glass				
1	1.30–1.50	1.43	1.15–1.40	1.27	0.16
3	1.63–2.44	2.00	1.19–1.45	1.29	0.71*
4	2.57–3.34	2.92	2.68–3.07	2.90	0.02
5	1.62–1.65	1.64	1.60–1.65	1.62	0.02
6	1.25–1.45	1.33	1.45–1.55	1.50	−0.17
7	2.67–2.93	2.77	2.54–2.99	2.74	0.03
8	1.02–1.07	1.04	0.97–1.11	1.02	0.02
9	3.32–3.60	3.44	3.32–3.77	3.50	−0.06
Average	—	2.07	—	1.98	0.09
	Sample 10. Basic Slag				
1	3.05–3.05	3.05	3.00–3.05	3.02	0.03
3	3.14–3.16	3.15	3.10–3.12	3.11	0.04
4	3.01–3.01	3.01	2.95–2.95	2.95	0.06*
5	3.10–3.12	3.11	3.00–3.12	3.07	0.04
6	3.15–3.25	3.20	3.20–3.20	3.20	0.00
7	3.08–3.11	3.10	3.16–3.19	3.18	−0.08*
9	3.17–3.25	3.21	3.10–3.15	3.12	0.09*
Average	—	3.12	—	3.09	0.03*
	Sample 11. Basic Slag				
1	1.60–1.65	1.62	1.50–1.50	1.50	0.12*
3	1.55–1.57	1.56	1.72–1.73	1.73	−0.17*
4	1.51–1.55	1.53	1.44–1.56	1.47	0.06
5	1.57–1.62	1.60	1.50–1.52	1.51	0.09*
6	1.60–1.75	1.68	1.60–1.65	1.62	0.06
7	1.60–1.61	1.60	1.55–1.60	1.58	0.02
8	1.48–1.59	1.52	1.50–1.55	1.52	0.00
9	2.07–2.20	2.12	1.50–1.50	1.50	0.62*
Average	—	1.65	—	1.55	0.10*
	Sample 12. Phosphate Rock				
1	27.40–27.70	27.60	27.40–27.60	27.53	0.07
3	28.01–28.01	28.01	27.50–27.57	27.55	0.46*
4	26.91–27.01	26.96	26.61–26.71	26.65	0.31*
5	27.75–27.88	27.84	27.40–27.55	27.50	0.34*
6	27.25–27.70	27.52	27.55–27.70	27.63	−0.11
7	27.65–27.70	27.68	27.65–27.81	27.74	−0.06
9	28.00–28.25	28.15	27.70–27.80	27.75	0.40*
Average	—	27.68	—	27.48	0.20*

TABLE 3.—(*continued*)

COLLABORATOR	CITRATE-INSOLUBLE P_2O_5, BY—				DIFFERENCE IN AVERAGE RESULTS[c]
	METHOD I[a]		METHOD II[b]		
	RANGE	AVERAGE	RANGE	AVERAGE	
	per cent	*per cent*	*per cent*	*per cent*	
		Sample 13. Superphosphate			
1	0.05–0.10	0.07	0.05–0.05	0.05	0.02
3	0.09–0.11	0.10	0.10–0.11	0.11	−0.01
4	0.09–0.09	0.09	0.06–0.10	0.08	0.01
5	0.06–0.07	0.06	0.06–0.08	0.07	−0.01
6	0.10–1.15	0.12	0.10–0.15	0.12	0.00
7	0.05–0.07	0.06	0.04–0.06	0.05	0.01
8	0.05–0.07	0.06	0.06–0.08	0.07	−0.01
9	0.12–0.15	0.13	0.10–0.10	0.10	0.03
Average	—	0.09	—	0.08	0.01
		Sample 14. Superphosphate			
7	1.02–1.08	1.05	1.07–1.15	1.11	−0.06

RESULTS OF ANALYSIS

Table 3 summarizes the results reported by the collaborators for citrate-insoluble P_2O_5 in the samples, as determined by the official method and by continuous agitation in the MacIntire-Marshall-Meyer apparatus. The results obtained by other methods of agitation during the citrate digestion are shown in Table 4.

COMMENTS OF COLLABORATORS

N. S. Chapman.—The citrate-insoluble residues were washed with water at 65°C. Phosphorus was determined volumetrically using solutions standardized against National Bureau of Standards phosphate rock. In a number of cases, measurements were made of the temperature of the liquid in the flasks at the end of the digestion period, in relation to the position of the flask in the bath or chamber. The range in variation from 65°C. was: official method, 0 to +1°; MacIntire-Marshall-Meyer apparatus, 0 to +3°; Ross-Kershaw apparatus, −3 to +1°; continuous stirring, −3 to +3°.

K. L. Elmore.—The following observations were made in carrying out these analyses: The lowering of the temperature of the air bath during introduction of samples in the continuous shaking procedure (MacIntire-Marshall-Meyer apparatus) presents a serious problem. We placed 9 flasks containing 100 ml. of the citrate solution in the bath and brought the temperature of the bath and solutions up to 65°C. as specified. In adding the sample charges to the solutions the temperature dropped to 58°C. due to opening the doors of the bath to introduce the charges. Fifty minutes of the 1-hour shaking period were consumed before the bath again reached 65°C. To obviate this difficulty the same samples were rerun by preheating the solutions to 65°C. in flasks set in a water bath. This procedure resulted in a tem-

TABLE 4.—*Effect of different methods of agitation during citrate digestion on results for citrate-insoluble P_2O_5*

COLLABORATOR	CITRATE-INSOLUBLE P_2O_5 BY METHOD				DIFFERENCE IN RESULTS BY METHOD I AND METHOD—		
	I[a]	II[b]	III[c]	IV[d]	II[e]	III[e]	IV[e]
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
	Sample 1. Ammoniated Superphosphate						
2	1.65	—	—	1.23	—	—	0.42*
3	0.87	0.62	0.73	0.82	0.25*	0.14	0.05
7	1.75	1.67	1.78	1.69	0.08	−0.03	0.06
	Sample 2. Ammoniated Superphosphate						
2	2.44	—	—	1.93	—	—	0.51*
3	2.03	1.57	1.53	1.46	0.46*	0.50*	0.57*
7	2.65	3.03	2.95	2.77	−0.38	−0.30*	−0.12
	Sample 3. Mixed Fertilizer						
2	0.31	—	—	0.20	—	—	0.11*
3	0.19	0.19	0.19	0.21	0.00	0.00	−0.02
7	0.13	0.28	0.31	0.18	−0.15*	−0.18*	−0.05
	Sample 4. Mixed Fertilizer						
2	0.28	—	—	0.28	—	—	0.00
3	0.29	0.20	0.20	0.22	0.09*	0.09*	0.07*
7	0.29	0.34	0.39	0.35	−0.05	−0.10*	−0.06
	Sample 5. Dicalcium Phosphate						
2	0.63	—	—	0.66	—	—	−0.03
3	0.63	0.62	0.61	0.62	0.01	0.02	0.01
7	0.58	0.73	0.65	0.74	−0.15*	−0.07	−0.16*
	Sample 6. Fused Alpha Phosphate						
2	5.09	—	—	4.82	—	—	0.27
3	5.19	4.77	4.84	4.87	0.42*	0.35*	0.32*
7	5.44	5.09	5.25	4.89	0.35*	0.19*	0.55*
	Sample 7. Sintered Alpha Phosphate						
2	1.49	—	—	1.74	—	—	−0.25*
3	1.60	1.53	1.54	1.56	0.07	0.06*	0.04*
7	1.70	1.66	1.71	1.69	0.04	−0.01	0.01
	Sample 8. Calcined Alpha Phosphate						
2	3.24	—	—	3.18	—	—	0.06
3	3.34	3.10	3.15	3.13	0.24*	0.19*	0.21*
7	3.43	3.50	3.50	3.28	−0.07	−0.07	0.15*

[a] Official; manual shaking at 5-minute intervals.

[b] Continuous end-over-end rotation in MacIntire-Marshall-Meyer apparatus (11).

[c] Continuous shaking in horizontal plane in Ross-Kershaw apparatus (18).

[d] Continuous stirring.

[e] The minus sign denotes that the result by this method is higher than that by method I. The asterisk denotes that the difference is statistically significant at the 5% level.

TABLE 4.—(*continued*)

COLLABORATOR	CITRATE-INSOLUBLE P_2O_5 BY METHOD I[a]	II[b]	III[c]	IV[d]	DIFFERENCE IN RESULTS BY METHOD I AND METHOD— II[e]	III[e]	IV[e]
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
				Sample 9. Phosphate Rock-Magnesium Silicate Glass			
2	1.56	—	—	1.66	—	—	−0.10
3	2.00	1.29	1.72	1.28	0.71*	0.28	0.72*
7	2.77	2.74	2.65	2.96	0.03	0.12	−0.19
				Sample 10. Basic Slag			
2	3.16	—	—	3.37	—	—	−0.21
3	3.15	3.11	3.12	3.12	0.04	0.03*	0.03*
7	3.10	3.18	3.12	3.24	−0.08*	−0.02	−0.14*
				Sample 11. Basic Slag			
2	1.71	—	—	1.70	—	—	0.01
3	1.56	1.73	1.73	1.73	−0.17*	−0.17*	−0.17*
7	1.60	1.58	1.56	1.69	0.02	0.04	−0.09*
				Sample 12. Phosphate Rock			
2	26.56	—	—	26.50	—	—	0.06
3	28.01	27.55	27.91	27.67	0.46*	0.10*	0.34*
7	27.68	27.74	27.92	27.45	−0.06	−0.24*	0.23*
				Sample 13. Superphosphate			
2	0.09	—	—	0.07	—	—	0.02
3	0.10	0.11	0.10	0.09	−0.01	0.00	0.01
7	0.06	0.05	0.01	0.10	0.01	0.05	−0.04
				Sample 14. Superphosphate			
7	1.05	1.11	1.07	1.23	−0.06	−0.02	−0.18*

perature drop of only 2°C., which was regained in the first 5 minutes of the 1-hour shaking period. The following table shows the effects of the two techniques.

SAMPLE	MATERIAL	CITRATE-INSOLUBLE P_2O_5 BY PREHEATING SOLUTION IN— WATER BATH	DIGESTION CHAMBER
		per cent	*per cent*
1	Ammoniated superphosphate	1.54	1.87
2	Ammoniated superphosphate	1.78	2.48
3	Mixed fertilizer	0.08	0.12

We also determined P_2O_5 insoluble in 2 per cent citric acid (Wagner method) on the more basic materials for comparison with the official citrate method, with the following results:

SAMPLE	MATERIAL	P_2O_5 INSOLUBLE IN—	
		CITRATE	CITRIC ACID
		per cent	per cent
9	Phosphate rock-magnesium silicate glass	2.92	0.00
10	Basic slag	3.01	1.40
11	Basic slag	1.53	0.77

L. J. Hardin and H. S. Johnson, Jr.—All preliminary leachings of samples, filtrations of ammonium citrate digestates, and of "yellows" were made by suction, using the Shimer filter. All ammonium citrate digestates were washed with 5 per cent ammonium nitrate at 65°C. All phosphorus determinations were made volumetrically using solutions standardized against National Bureau of Standards phosphate rock. The temperature of our cabinet fluctuates ±1° when set for 65°C. and varies ±1.5° when set for 50°. It is considered that the values for some of the samples, namely 3, 4, and 13, are too small for consideration in the evaluation of the two methods.

R. M. Pinckney and L. F. Rader, Jr.—The citrate-insoluble residues were washed with water at 65°C. Phosphorus was determined volumetrically using solutions standardized against National Bureau of Standards phosphate rock. A comparison was made of the results for insoluble P_2O_5 in the samples as determined by the official citrate and citric acid methods, respectively. The data are given in the following table which also shows the *p*H values of the solutions at the end of the citrate digestion.

SAMPLE	MATERIAL	P_2O_5 INSOLUBLE IN—		*p*H OF CITRATE SOLUTION AFTER DIGESTION[c]
		CITRATE[a]	CITRIC ACID[b]	
		per cent	per cent	
1	Ammoniated superphosphate	1.75	0.38	7.00
2	Ammoniated superphosphate	2.65	0.47	7.05
3	Mixed fertilizer	0.13	0.29	7.10
4	Mixed fertilizer	0.29	0.24	7.10
5	Dicalcium phosphate	0.58	1.32	7.15
6	Fused alpha phosphate	5.44	4.25	7.65
7	Sintered alpha phosphate	1.70	1.15	7.50
8	Calcined alpha phosphate	3.43	2.56	7.95
9	Phosphate rock-magnesium silicate glass	2.77	0.38	8.20
10	Basic slag	3.10	2.87	8.12
11	Basic slag	1.60	1.34	8.00
12	Phosphate rock	27.68	23.57	7.05
13	Superphosphate	0.06	0.47	6.90
14	Superphosphate	1.05	2.34	6.85

[a] Official method; manual shaking at 5-minute intervals.
[b] With samples 1–4 and 13–14 the determinations were made on the water-insoluble residues.
[c] In a blank determination no change occurred in the *p*H value (6.98) of the ammonium citrate solution during the digestion period.

North Carolina Department of Agriculture.—Experience in this laboratory has been that the constant temperature, continuous agitation (end-over-end rotation) cabinet now on the market, embodies a number of difficulties which offset its possible advantages. One of the major difficulties is preheating. It has been observed here that when a set of flasks is put into the cabinet for processing, the temperature of the cabinet promptly rises to the desired level and so registers on the cabinet thermometer. It has been further found, however, that the actual solutions in the flasks do not reach the proper temperature until approximately 40 or 50 minutes have elapsed. This trouble indicates the need for a convenient, quick preheating arrangement. Other difficulties are the trouble and liability of handling flasks inverted and with the stopper downward. The spring cups and pistons for holding the flasks in place unfortunately in the present design are also excellent stopper pullers. There is always the high possibility that stoppers will be pulled out of the flasks when removing them from the cabinet.

INTERPRETATION OF RESULTS

Official Method vs. Continuous End-Over-End Rotation.—Among the 99 comparisons (averages of replicate determinations) between the official method (shaking at 5-minute intervals) and continuous end-over-end rotation in the MacIntire-Marshall-Meyer apparatus (Table 3), 69 (70 per cent) show lower results for citrate-insoluble P_2O_5 by continuous agitation, 21 by the official method, and 9 show no difference. The distribution of the differences, without regard for signs, is as follows:

DIFFERENCE	COMPARISONS
per cent	*number*
<0.05	41
0.05–0.09	20
0.10–0.14	9
0.15–0.24	10
0.25–0.49	15
0.62–1.40	4
0.00–1.40	99

Of the 29 individual comparisons that differ by more than 0.14 per cent P_2O_5, 23 (79 per cent) are in the direction of lower results with continuous agitation. Among the comparisons which show such differences, 22 (76 per cent) occur in the determinations on the samples of ammoniated superphosphate (Nos. 1 and 2), fused alpha phosphate (No. 6), phosphate rock-magnesium silicate glass (No. 9), and phosphate rock (No. 10). With 2 exceptions, all of the differences in excess of 0.24 per cent P_2O_5 occur in the results for these samples. These samples contain more than 1 per cent citrate-insoluble P_2O_5. On the other hand, the samples of sintered alpha phosphate (No. 7), calcined alpha phosphate (No. 8), and basic slag (Nos. 10 and 11) also contain more than 1 per cent citrate-insoluble P_2O_5, yet the differences between the 30 individual comparisons on these materials exceed 0.10 per cent P_2O_5 in only 6 instances.

For the 14 individual samples (Table 3), the average results of all the collaborators show lower values with continuous agitation in 12 instances. The greatest differences (0.11–0.48 per cent P_2O_5) in the average results, all in the direction of lower values for insoluble P_2O_5 with continuous agitation, occur in the comparisons on the ammoniated superphosphates, fused alpha phosphate, and phosphate rock.

Separate statistical analyses of the data for each sample were made by standard analysis of variance procedures, and the differences required for significance at the 5 per cent level were determined. The statistically significant differences are indicated by asterisks in the last column of Table 3.

Among the 99 comparisons (Table 3), only 39 show significant differences between the results for citrate-insoluble P_2O_5 by the two methods of agitation. Of these 39 comparisons, 33 (85 per cent) show differences significantly in favor of lower values for citrate-insoluble P_2O_5 by continuous agitation, and 16 of these significant differences occur in the 29 comparisons on the samples of ammoniated superphosphate, fused alpha phosphate, and phosphate rock.

The average results on the 13 individual samples analyzed by more than one collaborator (Table 3) show statistically significant differences in 9 instances, all in favor of lower values by continuous agitation. It will be noted, however, that these differences exceed 0.10 per cent P_2O_5 in the case of only the ammoniated superphosphates, the fused alpha phosphate, and the phosphate rock.

MacIntire, Marshall, and Meyer (11) state that better reproducibility of values for citrate-insoluble P_2O_5 are obtained with their apparatus (continuous end-over-end rotation in a constant-temperature chamber) than with shaking at 5-minute intervals as prescribed in the official method. While the data of Table 3 are not conclusive, they indicate a decided trend in that direction. With both procedures the greatest variations in the replicate determinations occur in the results on the phosphate rock-magnesium silicate glass (Sample 9). Several of the collaborators reported much difficulty in the analysis of this sample. Hill *et al.* (8) state that this type of material shows a marked tendency to cake when it is added to neutral ammonium citrate solution and that it is necessary to shake the flask vigorously immediately after addition of the sample to the solvent.

As previously mentioned, Elmore (Collaborator 4) reported that with the MacIntire-Marshall-Meyer apparatus markedly lower results for insoluble P_2O_5 in certain samples were obtained when the citrate solution was preheated to 65°C. in a water bath, than when the solution was preheated in the digestion chamber itself. This was attributed to the greater time required for the temperature of the chamber to return to 65°C. in the latter case. Although no special study was made of this factor, comparison of the results of Collaborators 1, 3, 4, 5, and 7, who preheated the solution in a water bath, with those of Collaborators 6, 8, and 9, who preheated the solution in the digestion chamber, gives no definite indication that this variation in technique contributed to the differences in the values obtained. It is important, nevertheless, that the operation be performed with the least possible disturbance of the chamber temperature.

L. J. Hardin and W. H. MacIntire have discussed "The Effect of Open vs. Closed Flasks in the Digestion of Phosphate Fertilizers with Neutral Ammonium Citrate" in a private communication to the Associate Referee. They write as follows:

"The effect of the retention of the NH_3 generated within the system was tested through digestions of duplicate charges of a 'calcined alpha phosphate' under two conditions, *i.e.*, (1) with the flask stoppered tightly during the period of shaking and digestion, and (2) the stopper removed after each shaking and then placed loosely during the intervals of the quiescent digestion. After 60-minute digestions the *p*H values of the respective filtrates were measured and the citrate-insoluble P_2O_5 contents determined."

CLOSED DIGESTION[a]			OPEN DIGESTION[b]	
INTERMITTENT SHAKING		CONTINUOUS SHAKING	INTERMITTENT SHAKING	
INSOLUBLE P_2O_5	*p*H OF FILTRATE	INSOLUBLE P_2O_5	INSOLUBLE P_2O_5	*p*H OF FILTRATE
per cent		*per cent*	*per cent*	
2.95	8.05	2.75	2.90	8.05
2.90	8.10	2.70	2.85	8.05

[a] Solvent brought to 65°C., sample introduced, and flask kept tightly stoppered throughout digestion and shakings.

[b] Stopper removed after each shaking and then replaced loosely until next shaking.

"The insoluble P_2O_5 values are in close agreement, whether the flask was tightly stoppered throughout or closed loosely between shakings to allow escape of some of the generated NH_3. A similar agreement was true of the final *p*H values. When the digestions were agitated continuously in the constant temperature cabinet, the citrate-insoluble values were somewhat lower than those obtained by means of the intermittent manual shaking."

Official Method vs. Other Methods of Agitation.—In Table 4 the average results for citrate-insoluble P_2O_5 obtained by shaking at 5-minute intervals (Method I, official) are compared, respectively, with those obtained by continuous end-over-end rotation in the MacIntire-Marshall-Meyer apparatus (Method II), continuous shaking in the horizontal plane in the Ross-Kershaw apparatus (Method III), and continuous stirring (Method IV). The differences required for significance at the 5 per cent level were computed in the same manner as for the data in Table 3. The following tabulation shows the number of comparisons and the average differences between the results by Method I and the other methods, for all the collaborators and all the samples. The data indicate that the results obtained by continuous shaking in the Ross-Kershaw apparatus agree somewhat more closely with those by the official method than do the results obtained either by continuous end-over-end rotation or by continuous stirring.

METHOD I VS.	COMPARISONS			
	TOTAL		SHOWING SIGNIFICANT DIFFERENCES	
	NUMBER	AVERAGE DIFFERENCE[a]	NUMBER	AVERAGE DIFFERENCE[a]
		per cent		*per cent*
Method II	27	0.08	13	0.16
Method III	27	0.04	13	0.04
Method IV	40	0.08	20	0.16

[a] The differences are in the direction of higher results for citrate-insoluble P_2O_5 by Method I.

CONCLUSIONS

Continuous agitation during the citrate digestion tends to give lower values for citrate-insoluble P_2O_5 than does shaking at 5-minute intervals as prescribed in the official method. The differences between the results by the two methods of agitation tend to be larger in the case of samples that are relatively high in citrate-insoluble P_2O_5. In general, however, these differences are much smaller than the differences in the results obtained on a given sample by different analysts using the same method of agitation. Variation in the method of continuous agitation does not appear to be an important factor.

From the analyst's standpoint, it appears that continuous agitation in an apparatus provided with means for automatic temperature control has the advantage over manual periodic agitation in that it conserves time, requires less attention, and tends to improve the reproducibility of the results.

The results of the present study, together with those of previous investigations, indicate that mechanical continuous agitation during the citrate digestion is worthy of adoption by this Association as an alternative to the present official procedure of manual intermittent agitation.

B. EFFECT OF SULFATE ON DETERMINATION OF P_2O_5 BY THE VOLUMETRIC METHOD

The interference of sulfate in the volumetric determination of P_2O_5, whereby high results are obtained when the ammonium phosphomolybdate precipitation is made at elevated temperatures, has been investigated by a number of workers (2, 3, 5, 14, 15, 16, 19). The subject was studied collaboratively by W. H. Ross, the Associate Referee on Phosphoric Acid, and the results were reported at the 1928 and 1929 meetings of this Association (15, 16). On the basis of this study the Association adopted two procedures for the volumetric determination of P_2O_5 (1, pp. 22–23) applicable, respectively, in the presence and the absence of sulfates. The first of these procedures involves precipitation of the phosphomolybdate at 25–30°C. with constant stirring or shaking for 30 minutes, while the second specifies precipitation at 45–50°C. and the maintenance of the precipitate at such temperature for 30 minutes with occasional stirring.

The fact that most fertilizers contain considerable sulfate usually restricts the analyst to the first of these procedures in the volumetric determination of P_2O_5 in such materials. As this necessitates the use of a stirring or shaking apparatus which may not be readily available to the analyst the provision of a volumetric procedure not requiring the use of such apparatus, yet applicable to the analysis of sulfate-containing materials, seems desirable. Furthermore, some analysts prefer, for various reasons, to precipitate the phosphomolybdate at 45–50°C. instead of 25–30°.

In 1907, Richardson (14) recommended that barium chloride be added immediately after completion of the acid digestion of the sample, in order to precipitate the sulfate and to eliminate its subsequent interference in the volumetric determination of P_2O_5 by the hot precipitation procedure. Breckenridge (3) recommended a similar modification but with the use of barium nitrate instead of the chloride. Also, elimination of sulfate interference by precipitation as the barium salt has been studied briefly by Shuey (19). Recently, C. C. Howes and A. T. Blackwell[3] have reported that very satisfactory results in the volumetric determination of P_2O_5 in sulfate-containing fertilizers by hot (45–50°) precipitation of the phosphomolybdate are obtained if the sulfate is first removed by means of 10 per cent barium chloride solution.

The present report summarizes the results secured in a collaborative study of the volumetric method for P_2O_5, with special reference to elimination of sulfate interference in the precipitation of the phosphomolybdate at 45–50°.

Two ordinary superphosphates, designated as Sample 13 and Sample 14 and made, respectively, from Florida pebble and Tennessee brown-rock phosphates, were submitted to the collaborators. These were well-cured, run-of-pile, commercial materials. Gravimetric determinations of P_2O_5 and SO_3 gave the following results:

SAMPLE	P_2O_5	SO_3
	per cent	*per cent*
13	20.77	32.06
14	20.40	31.87

COLLABORATORS' DIRECTIONS FOR ANALYSIS

1. Determine total P_2O_5 in each of the samples as directed in *Methods of Analysis, A.O.A.C.*, 6th ed., 1945, volumetric method, pp. 22–23, sec. **2.10** and **2.12(a)**, and sec. **2.10** and **2.12(b)**. Prepare the solutions of the samples as directed in sec. **2.8(a)** or in sec. **2.8(b)**, pp. 21–22. (Certain of the collaborators were instructed to

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[3] The Davison Chemical Corporation, Baltimore, Md. Private communications.

make the determinations on solutions prepared by method **2.8(a)**, others by method **2,8(b)**, and two by both methods.)

2. Repeat the determinations on solutions prepared as follows: Proceed as directed in sec. **2.8(a)** or **2.8(b)** to the point where digestion of the sample is completed, then add 20 ml of 10 per cent barium chloride solution and boil for a few minutes. Cool the solution and proceed with the determinations as before.

3. As it is desired to subject the results to statistical analysis, it is requested that for each sample the determinations be made simultaneously on solutions prepared in triplicate with and without addition of barium chloride. This will require a total of twelve determinations on each sample, *i.e.*, three determinations under each of the four sets of conditions as to use of barium chloride and as to temperature at which the phosphomolybdate is precipitated. It is necessary that the results of each determination be reported individually.

4. Your comments and observations concerning this investigation are requested, especially regarding the effect of precipitation temperature on the ease of filtering and washing the phosphomolybdate.

COLLABORATORS

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7. Dunn, Alice, Intern. Minerals & Chem. Corp., East Point, Ga.
8. Few, S. J., Miss. State Chem. Lab., State College, Miss.
9. Gilbert, Roland, R. I. Agr. Expt. Sta., Kingston, R. I.
10. Green, U. P., Dept. Agr., Ottawa, Canada.
11. Koch, R. C., and Pearce, T. J., Swift & Co., Plant Food Div., Hammond, Ind.
12. Lang, P. A., and Tosh, Ruth D., The Am. Agr. Chem. Co., Carteret, N. J.
13. Leslie, E. E., The Clemson Agr. Coll., Clemson, S. C.
14. Marshall, H. L., Southern Acid & Sulphur Co., Inc., Little Rock, Ark.
15. Montague, H. S., Miss. State Chem. Lab., State College, Miss.
16. Morgan, W. A., E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.
17. McAllister, Wm., Cooperative Mills, Inc., Baltimore, Md.
18. Rader, L. F., Jr., Bur. Plant Ind., Soils, and Agr. Eng., Beltsville, Md.
19. Rogers, C. J., Mass. Agr. Expt. Sta., Amherst, Mass.
20. Ryder, W. A., F. S. Royster Guano Co., Norfolk, Va.
21. Shuey, P. McG., Shuey & Co., Inc., Savannah, Ga.

RESULTS OF ANALYSIS

Table 5 summarizes the collaborators' results (averages of triplicate analyses) for total P_2O_5 in the samples, as determined by the volumetric procedures with: (1) precipitation of the phosphomolybdate at 25–30°C. and constant stirring or shaking for 30 minutes, without prior removal of sulfate (official); (2) the same, with prior removal of sulfate; (3) precipitation of the phosphomolybdate at 45–50°C. and occasional stirring during the 30-minute period of heating at such temperature, without prior removal of sulfate; and (4) the same, with prior removal of sulfate.

TABLE 5.—*Results for total P_2O_5 in superphosphate as affected by removal of sulfate prior to precipitation of ammonium phosphomolybdate at 25–30°C. and 45–50°C. in the volumetric procedure*

COLLAB-ORATOR	SULFATE PRESENT			SULFATE REMOVED			COL. VI MINUS COL. II
	25–30°C.[a]	45–50°C.[b]	COL. III MINUS COL. II	25–30°C.[a]	45–50°C.[b]	COL. VI MINUS COL. V	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
I	II	III	IV	V	VI	VII	VIII
A. Solution of Sample by Method 2.8(a)[c]							
SAMPLE 13							
1	20.86	21.08	0.22	20.80	20.93	0.13	0.07
4	20.95	21.32	0.37	20.87	20.92	0.05	−0.03
6	20.88	21.75	0.87	20.78	21.07	0.29	0.19
7	20.73	21.18	0.45	20.68	20.87	0.19	0.14
8	21.00	21.60	0.60	21.00	20.90	−0.10	−0.10
10	21.30	21.87	0.57	21.07	21.30	0.23	0.00
13	21.08	20.98	−0.10	20.48	20.47	−0.01	−0.61
15	20.91	21.41	0.50	20.95	20.79	−0.16	−0.12
16	20.93	20.92	−0.01	20.81	20.83	0.02	−0.10
18	20.87	21.34	0.47	20.92	21.08	0.16	0.21
19	20.65	21.09	0.44	20.67	20.90	0.23	0.25
20	20.83	20.83	0.00	20.77	20.77	0.00	−0.06
Av.	20.92	21.28	0.36	20.82	20.90	0.08	−0.02
Min.	20.65	20.83	−0.10	20.48	20.47	−0.16	−0.61
Max.	21.30	21.87	0.87	21.07	21.30	0.29	0.25
SAMPLE 14							
1	20.46	20.58	0.12	20.42	20.46	0.04	0.00
4	20.45	20.65	0.20	20.42	20.47	0.05	0.02
6	20.30	21.14	0.84	20.21	20.41	0.20	0.11
7	20.21	20.57	0.36	20.18	20.37	0.19	0.16
8	20.40	21.08	0.68	20.40	20.40	0.00	0.00
10	20.73	21.50	0.77	20.73	20.90	0.17	0.17
13	20.97	21.00	0.03	20.42	20.58	0.16	−0.39
15	20.41	20.86	0.45	20.31	20.40	0.09	−0.01
16	20.61	20.48	−0.13	20.44	20.51	0.07	−0.10
18	20.43	20.84	0.41	20.43	20.53	0.10	0.10
19	20.14	20.32	0.18	20.13	20.20	0.07	0.06
20	20.40	20.47	0.07	20.40	20.40	0.00	0.00
Av.	20.46	20.79	0.33	20.37	20.47	0.10	0.01
Min.	20.14	20.32	−0.13	20.13	20.20	0.00	−0.39
Max.	20.97	21.50	0.84	20.73	20.90	0.20	0.17

[a] With constant stirring or shaking.
[b] With occasional stirring.
[c] 30 ml. HNO_3 and 3–5 ml. HCl.
[d] 15–30 ml. HCl and 3–10 ml. HNO_3.
[e] Samples 13 and 14, solution method 2.8(a).
[f] Samples 13 and 14, solution method 2.8(b).
[g] Sample 13, solution methods 2.8(a) and 2.8(b).
[h] Sample 14, solution methods 2.8(a) and 2.8(b).
[i] All samples and all solution methods.

TABLE 5.—(*continued*)

COLLABORATOR	SULFATE PRESENT			SULFATE REMOVED			COL. VI MINUS COL. II
	25–30°C.[a]	45–50°C.[b]	COL. III MINUS COL. II	25–30°C.[a]	45–50°C.[b]	COL. VI MINUS COL. V	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
I	II	III	IV	V	VI	VII	VIII
			B. Solution of Sample by Method **2.8(b)**[d]				
			SAMPLE 13				
2	21.04	21.90	0.86	20.76	20.87	0.11	−0.17
3	20.81	21.12	0.31	20.71	20.81	0.10	0.00
5	20.72	21.09	0.37	20.25	20.61	0.36	−0.11
6	21.02	21.60	0.58	20.99	20.81	−0.18	−0.21
9	20.69	20.87	0.18	20.68	20.73	0.05	0.04
11	20.76	20.84	0.08	20.76	20.66	−0.10	−0.10
12	20.98	21.81	0.83	20.73	20.95	0.22	−0.03
14	21.54	21.84	0.30	21.55	21.51	−0.04	−0.03
17	20.80	20.96	0.16	20.39	20.84	0.45	0.04
18	20.99	21.40	0.41	20.82	21.01	0.19	0.02
21	21.01	21.10	0.09	20.96	20.97	0.01	−0.04
Av.	20.94	21.32	0.38	20.78	20.89	0.11	−0.05
Min.	20.69	20.84	0.08	20.25	20.61	−0.18	−0.21
Max.	21.54	21.90	0.86	21.55	21.51	0.45	0.04
			SAMPLE 14				
2	20.49	21.31	0.82	20.33	20.44	0.11	−0.05
3	20.26	20.56	0.30	20.24	20.24	0.00	−0.02
5	20.26	20.70	0.44	20.04	20.10	0.06	−0.16
6	20.52	21.02	0.50	20.47	20.27	−0.20	−0.25
9	20.19	20.42	0.23	20.26	20.28	0.02	0.09
11	20.33	20.48	0.15	20.22	20.24	0.02	−0.09
12	20.46	21.19	0.73	20.24	20.39	0.15	−0.07
14	20.79	20.96	0.17	20.91	20.84	−0.07	0.05
17	20.20	20.66	0.46	19.82	20.42	0.60	0.22
18	20.47	20.85	0.38	20.37	20.40	0.03	−0.07
21	20.47	20.58	0.11	20.32	20.36	0.04	−0.11
Av.	20.40	20.79	0.39	20.29	20.36	0.07	−0.04
Min.	20.19	20.42	0.11	19.82	20.10	−0.20	−0.25
Max.	20.79	21.31	0.82	20.91	20.84	0.60	0.22
			C. Grand Averages				
[e]	20.69	21.03	0.34	20.59	20.68	0.09	−0.01
[f]	20.67	21.05	0.38	20.53	20.62	0.09	−0.05
[g]	20.93	21.30	0.37	20.80	20.89	0.09	−0.04
[h]	20.43	20.79	0.36	20.33	20.41	0.08	−0.02
[i]	20.68	21.04	0.36	20.56	20.65	0.09	−0.03

COMMENTS OF COLLABORATORS

H. C. Batton.—It is my opinion that there is little, if anything, to choose between cold precipitation of ammonium phosphomolybdate without prior precipitation of sulfates, and precipitation at 45–50°C. after sulfates have been removed. In fact, for a period of almost 15 years, I have been determining total phosphate by sulfate removal and hot precipitation on presumably identical portions of samples which have been analyzed by another laboratory by cold precipitation without removal of sulfates. I estimate that, during that time, such comparative analyses have been made on about 2,000 samples, with very few instances in which significant differences occurred. In fact, only two of all those samples had to be sent to a referee for final settlement.

Charles Bollfrass.—As was expected, the samples precipitated at the higher temperature yielded larger particles which came down more rapidly and were more easily filtered and washed. However, the precipitates obtained at 25–30°C. were entirely manageable and satisfactory from the standpoint of filtering and washing ease. Elimination of the sulfate did not seem to affect the particle size of the precipitate. Commenting without knowledge of the true analyses, it would seem that either precipitation at 25–30°C. without prior removal of the sulfate or precipitation at 45–50°C. with prior removal of the sulfate, yields reproducible although slightly divergent results. In addition to the information requested, experiments were run to determine the effect of the rate of barium chloride addition. These results indicated that the speed of addition had little or no effect on the per cent total P_2O_5 determination at either temperature.

N. S. Chapman.—Filtering and washing was not facilitated by precipitating the phosphomolybdate at 45–50°C.

Alice Dunn.—No difference was noted in the ease of filtering and washing the phosphomolybdate in any of the precipitations. We use suction filters with paper pulp mats. A small error is introduced in the sulfate precipitation method by the displacing effect of the barium sulfate. However, it appears this error would change the P_2O_5 result only about 0.03–0.04 per cent.

S. J. Few and H. S. Montague.—No difficulty in filtering when sulfates were precipitated.

Roland Gilbert.—No differences were noticed in filtration and washing the yellow precipitate, nor were any difficulties encountered under any of the conditions. The yellow precipitate was filtered on Whatman No. 7 paper.

P. A. Lang and Ruth D. Tosh.—At 45–50°C. the solutions filtered more rapidly than at 25–30°C. with and without removal of sulfate. This, however, is of no real help, as we filter by gravity using a long-stem fluted funnel and a No. 40, 11 cm. Whatman filter paper which filters rapidly at both of the above temperature ranges.

E. E. Leslie.—At the temperature of 45–50°C. the "yellows" tend to cling to the Phillips beakers, making the washings more difficult. Also, they go through the filter pads more easily than at the lower temperature.

H. L. Marshall.—The solution, diluted with 50 ml. of water, was treated with barium chloride and boiled for 3 minutes. No effect was noticeable on the filtration of the phosphomolybdate. We use Moore-Shimer filters (Whatman No. 42 paper).

Wm. McAllister.—The temperature of precipitation did not seem to influence, to a very great degree, the amount of creeping of the precipitate.

W. A. Morgan.—The yellow precipitates were filtered in Shimer tubes through packed filter paper pulp (Whatman No. 1). Precipitates obtained at 45–50°C. seemed to filter more rapidly.

L. F. Rader, Jr.—Little or no difference in rate of filtering and ease of washing the phosphomolybdate precipitates was observed. More complete removal of the sulfate appears to be obtained by adding the barium chloride directly to the hot acid digestate than by diluting the digestate before adding the chloride. The amounts of sulfate remaining in solutions after treatment by adding barium chloride directly to the hot digestates were determined with the following results:

SAMPLE	TOTAL SO_3	SO_3 REMAINING AFTER $BaCl_2$ TREATMENT OF SOLUTION PREPARED BY METHOD			
		2.8(a)[a]		2.8(b)[b]	
		IN SOLUTION	PORTION OF TOTAL	IN SOLUTION	PORTION OF TOTAL
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
13	32.06	1.00	3.1	0.23	0.7
14	31.87	1.51	4.7	0.57	1.8

[a] 30 ml. HNO_3 and 3–5 ml HCl

[b] 15–30 ml. HCl and 3–10 ml. HNO_3.

C. J. Rogers.—No significant difference was observed in the ease of filtering or washing of precipitates by the different methods.

P. McG. Shuey.—Results are slightly lower when barium chloride is added both by the hot and cold precipitation methods, despite the fact that the precipitated barium sulfate is separated by filtration. It appears, therefore, that the barium sulfate formed has the tendency to drag down some P_2O_5. The regular official method appears more accurate.

INTERPRETATION OF RESULTS

With very few exceptions the data of the 46 comparisons given in Table 5, columns II and III, agree with those of previous investigators in showing that, in the presence of sulfate, precipitation of the phosphomolybdate at 45–50°C. leads to considerably higher values for P_2O_5 than does precipitation at 25–30°. Removal of the sulfate prior to hot precipitation of the phosphomolybdate usually brings the values into much closer agreement (compare columns IV and VIII). Under these conditions the values obtained by hot precipitation are actually lower than those by cold precipitation in slightly more than half of the 46 comparisons (column VIII).

With cold precipitation of the phosphomolybdate the values obtained when sulfate is removed (column V) are usually lower than those obtained when sulfate is not removed (column II). Likewise, in the collaborative studies conducted by Ross (15, 16) it was found that with cold precipitation the results on sulfate-free samples were nearly always lower than those on the same samples to which sulfate had been added. This would seem to indicate some interference by sulfate even when the precipitation is made at room temperature.

It will be noted that when sulfate is removed the values obtained with hot precipitation (column VI) are usually higher than those obtained with cold precipitation (column V). A similar trend in results has been reported by Ross (15, 16) on sulfate-free samples under like conditions of temperature.

With removal of sulfate the trend appears to be toward somewhat higher values for P_2O_5 when solution of the sample is effected by method 2.8(a) (30 ml HNO_3 and 3–5 ml HCl) as compared with solution by method 2.8(b) (15–30 ml HCl and 3–10 ml HNO_3), whether the phosphomolybdate is precipitated at 25–30° (column V) or 45–50° (column VI). For the same sample and precipitation temperature, however, the average difference in all results by the two methods of solution is small and is within the range of permissible variance.

Statistical analysis, by means of the standard analysis of variance procedure, was made of the combined results of each collaborator. Variations among the results of the replicate determinations by each collaborator were ignored, as these were generally very small in comparison with the differences among the average results obtained by the individual collaborators.

The results of this analysis indicate that the values obtained by precipitating the phosphomolybdate at 25–30° without first removing the sulfate are not significantly different at the 5 per cent level from those obtained when the sulfate is removed and the precipitation is then made at 45–50°. Likewise, removal of the sulfate does not affect the values significantly when the subsequent precipitation is made at 25–30°. With precipitation at 45–50°, on the other hand, removal of the sulfate results in much lower values, and the difference is significant at the 0.1 per cent level. Under comparable conditions, the values are not affected significantly by the method of dissolving the sample.

CONCLUSIONS

In the volumetric determination of P_2O_5 in sulfate-containing fertilizers, removal of the sulfate as the barium salt followed by precipitation of the phosphomolybdate at 45–50° with occasional stirring gives results which are generally in good agreement with those obtained by the official method in which the sulfate is not removed but the precipitation is made at 25–30° with constant stirring or shaking. Removal of the sulfate followed by hot precipitation of the phosphomolybdate has the advantage that it does not require the use of a stirring or shaking apparatus—equipment which may not be readily available to the analyst. The results of the present study indicate that this procedure is worthy of adoption by this Association as an alternative method for the determination of P_2O_5.

ACKNOWLEDGMENT

The Associate Referee wishes to express his appreciation of the splendid cooperation given by the collaborators and their respective organizations in the investigations covered by this report.

RECOMMENDATIONS*

It is recommended—

(1) That the first six sentences of *Methods of Analysis, A.O.A.C.*, 1945,

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* For report of Subcommittee A and action by the Association, see *This Journal*, 31, 42 (1948).

p. 24, sec. **2.16(a)**, acidulated samples, lines 1–11, be changed to read:

"After removing water-soluble P_2O_5, **2.13**, transfer the filter and residue, within a period not to exceed 1 hour, to 200 or 250 ml flask containing 100 ml NH_4 citrate soln previously heated to 65°. Close flask tightly with a smooth rubber stopper, shake vigorously until filter paper is reduced to pulp, relieve pressure by momentarily removing stopper, and proceed by one of the following methods: (1) Loosely stopper flask to prevent evaporation, place in water bath regulated to maintain contents of flask at exactly 65°, keep level of H_2O in bath above that of citrate soln in flask, and shake every 5 min.; (2) continuously agitate contents of stoppered flask by means of apparatus equipped to maintain contents of flask at exactly 65°. At expiration of exactly 1 hour from time filter and residue were introduced, remove flask from bath or apparatus and immediately filter contents as rapidly as possible through Whatman filter paper No. 5 or other paper of equal speed and retentiveness."

(2) That the present paragraph of *Methods of Analysis, A.O.A.C.*, 1945, p. 23, sec. **2.11**, preparation of solution, be designated as **(a)** and be terminated with the words "suitable solvent," and that a second paragraph be added to read:

"**(b)** *Not applicable in preparation of solns by sulfuric acid digestion.*—Proceed as directed under **2.8(a)**, **(b)**, or **(c)**, preferably **(a)** when these acids are a suitable solvent, to point where acid digestion of sample is completed. Add 25 ml of 10% $BaCl_2$ soln to the hot digestate, boil ca 2 min, and continue as directed under **2.8**."

(3) That sec. **2.12** of *Methods of Analysis, A.O.A.C.*, 1945, p. 23, be changed as follows:

(1) At the beginning of sec. **2.12(a)** add the sentence, "Prepare soln of sample as directed under **2.11(a)**."

(2) In sec. **2.12(b)** delete the phrase, "Not applicable to superphosphate and other fertilizers that contain sulfates (5)," and begin the section with the new sentence, "Prepare soln of sample as directed under **2.11(b)**."

(3) Add a new section to read:

"**(c)** *Not applicable to superphosphate and other fertilizers that contain sulfate or to solns prepared with the aid of sulfuric acid* (*5*).—Prepare soln of sample as directed under **2.11(a)**. Proceed as directed under **(b)**."

(4) That the other phosphate investigations recommended in the reports of the Referee on Fertilizers (6) and the Associate Referee on Phosphoric Acid (10) presented at the preceding meeting of this Association (October 14–16, 1946) be undertaken.

LITERATURE CITED

(1) *Methods of Analysis, A.O.A.C.*, 1945.
(2) Bible, C. M., *This Journal*, **11**, 126–28 (1928).
(3) Breckenridge, J. E., *Ind. Eng. Chem.*, **16**, 1180 (1924).
(4) Curtis, H. A., Copson, R. L., Brown, E. H., and Pole, G. R., *Ibid.*, **29**, 766–770 (1937).
(5) Falk, K. G., and Sugiura, K., *J. Am. Chem. Soc.*, **37**, 1507–15 (1915).
(6) Fraps, G. S., *This Journal*, **30**, 226–28 (1947).
(7) Hignett, T. P., and Hubbuch, T. N., *Ind. Eng. Chem.*, **38**, 1208–16 (1946).
(8) Hill, W. L., Ward, F. N., Armiger, W. H., and Jacob, K. D., *This Journal*, **31**, 381 (1948).

(9) Jacob, K. D., Rader, L. F., Jr., and Tremearne, T. H., *Ibid.*, **19**, 449–72 (1936).
(10) ——, Ward, F. N., Hill, W. L., and Pinkerton, C., *Ibid.*, **30**, 529–48 (1947).
(11) MacIntire, W. H., Marshall, H. L., and Meyer, T. A., *Ibid.*, **27**, 272–83 (1944).
(12) ——, Winterberg, S. H., Hatcher, B. W., and Palmer, G., *Soil Sci.*, **57**, 425–42 (1944).
(13) Reynolds, D. S., Jacob, K. D., and Rader, L. F., Jr., *Ind. Eng. Chem.*, **26**, 406–12 (1934).
(14) Richardson, W. D., *J. Am. Chem. Soc.*, **29**, 1314–15 (1907).
(15) Ross, W. H., *This Journal*, **12**, 170–76 (1929).
(16) ——, *Ibid.*, **13**, 202–07 (1930).
(17) ——, and Jacob, K. D., *Ibid.*, **20**, 231–49 (1937).
(18) ——, and Merz, A. R., In *Wiley's Principles and Practice of Agricultural Analysis*, 3d ed., vol. II, 1931, p. 178.
(19) Shuey, P. McG., *Ind. Eng. Chem.*, **9**, 367–70 (1917).
(20) Walthall, J. H., and Bridger, G. L., *Ind. Eng. Chem.*, **35**, 774–77 (1943).

REPORT ON MOISTURE IN FERTILIZERS

By W. L. Hill (Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Md.), *Associate Referee*

With his report on Moisture in Fertilizers in 1946 Dr. Ross closed his work on moisture. Death intervened before the report was presented for publication and the manuscript was not found among the deceased's papers. The single recommendation, it is recalled, provided for a collaborative study of the present A.O.A.C. method, the air-flow method and the vacuum-drying method for determining moisture. A paper read by Dr. Ross at the 1944 meeting reviewed his moisture work and discussed the performance of the air-flow and vacuum-drying methods on ammonium nitrate and other fertilizer materials, but did not describe the procedures for the methods. This paper and one on the air-flow method appeared in the November issue of the Association's *Journal*, Vol. 30, 1947.

In view of the publication status of previous work the consensus of opinion last year was that the recommended collaborative work should not be undertaken this year. At the same time the Referee on Fertilizers suggested that, if possible, exploratory work be done on the use of ether as an extractant for moisture and on the use of low-boiling azeotropes. It has not been possible to conduct the suggested work this year. Since the delay of one year has not lessened the need for a collaborative study of methods, the recommendation of last year is retained and the procedures proposed for the air-flow and vacuum-drying methods are attached as a part of this report.

RECOMMENDATION*

It is recommended that a collaborative study be made comparing the present A.O.A.C. method with the air-flow and vacuum-drying methods for determining free moisture in fertilizer materials and mixed fertilizers.

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* For report of Subcommittee A and action by the Association, see *This Journal*, **31**, 42 (1948).

FREE MOISTURE IN FERTILIZERS

Air-flow Method

APPARATUS

(A) *Manifold Assembly.*—A metal box (Fig. 1) 10½×2½×1¼ inches in size is equipped with a ¼-inch nipple centrally located on one side for attachment to a vacuum line, and 6 1¼-inch tapered stopper seats evenly spaced along the top to accommodate No. 6, one-hole, rubber stoppers. A 1¼-inch length of light metal tubing, ½ inch in diameter, extends through each rubber stopper to a height of ¼ inch above the surface for the purpose of centering a fritted glass crucible over the hole in the stopper. Since the crucible is held in place by suction, it is necessary to grind a smooth surface on the lower edge of each fritted glass crucible and on the surface of the stopper in order to insure an air-tight connection between the edge of the crucible and the stopper when air is being drawn through the sample in the crucible.

(B) *Crucibles.*—Pyrex glass, approximately 1¾ inches tall, 1 $\frac{3}{16}$ inches in diameter and having a ⅞-inch fine-porosity fritted glass plate. Individual crucibles of a set should all have approximately the same porosity. A matched set may be obtained by selecting several that pass a given quantity of air at constant pressure in approximately the same length of time.

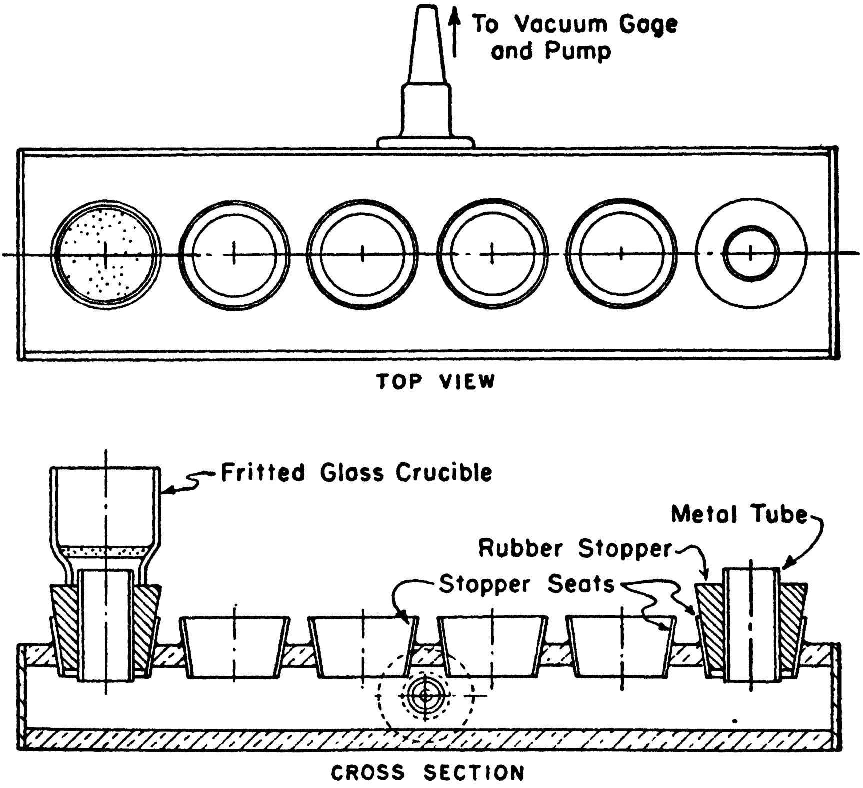

FIG. 1.—Manifold for Use in Determining Moisture by the Air-flow Method

(C) *Vacuum Gage.*—A standard instrument for insertion in the rubber vacuum line between the source of suction and the manifold.

(D) *Constant Temperature Oven.*—A standard laboratory oven, preferably of the type vented so that incoming air passes directly over the heating coils.

DETERMINATION

Weigh 2 g of prepared sample, 2.2, in a tared, fritted glass crucible. (Extremely hygroscopic or damp materials should be weighed out of difference in covered crucibles.) Place crucible on manifold in the oven at 60°. Aspirate for 2 hours under 15 in. of vacuum. Cool in desiccator, for 30 min. and reweigh. Calculate percentage loss in weight.

Vacuum-drying Method

DETERMINATION

Place 4 g of prepared sample, 2.2, in a short-type, tared weighing dish of a size not less than 2 in. in diameter. (Extremely hygroscopic or damp materials should be weighed out of difference in covered dishes.) Place in a vacuum desiccator over anhydrous magnesium perchlorate for 16 hours under not less than 25 in. of vacuum. Reweigh and calculate percentage loss in weight.

NOTE: A drying period of 16 hrs. represents over-night drying. The average type of fertilizer will release its free moisture in 3–6 hrs. However, over-night drying insures best results, especially on very damp materials and on samples containing high amounts of adsorbed water.

REPORT ON NITROGEN IN FERTILIZERS

By M. P. ETHEREDGE (Mississippi State Chemical Laboratory, State College, Mississippi), *Associate Referee*

Last year, A. L. Prince pointed out in his report to the Association (*This Journal*, **30**, 228, 1947) the importance of better agreements on the determination of ammonium nitrate. He was somewhat disappointed that so few of his collaborators had tried the Devarda procedure. Therefore, it was recommended that further collaborative work be done on methods of determining nitrogen in ammonium nitrate, with special reference to the Devarda alloy method.

It was further called to the attention of the Association that fertilizers with high nitrates and chlorides were difficult to check, and quite often the nitrogen content was reported as being far lower than the theoretical amount present.

With this in mind, three samples were selected for analysis. Sample No. 1 was a pure ammonium nitrate. Sample No. 2 was a commercial grade of ammonium nitrate. Sample No. 3 contained a large amount of sodium nitrate and potassium chloride.

Samples Nos. 1 and 2 were to be analyzed by the Devarda procedure, distilled with magnesium oxide and, also, with a small amount of sodium hydroxide. Collaborators were asked to use a Corning bulb of the type No. 2020 or No. 2040. This latter bulb, the Iowa type, was included because some laboratories use it. It has been the experience of the Associate Referee over a period of years that this bulb will produce a higher and less consistent blank than the other type. Collaborators were asked to use a blank.

Owing to the rapidly growing interest in the formaldehyde-titration procedure, collaborators were later asked to use this procedure for the two samples of ammonium nitrate which were sent out for study.

Collaborators were asked to determine nitrogen in the mixed fertilizer, sample No. 3, by the Kjeldahl method, **2.27**. Also enclosed was a copy of the procedure suggested to the Association by Mr. Shuey last fall. Later, the collaborators were asked to try the method of Dyer and Hamence, [*Analyst*, **63**, 866 (1938)].

The samples were sent to twenty-six collaborators. The Associate Referee is grateful to those reporting their results in time to make this report possible. Also, we are indebted to J. W. MacKay, Chief Chemist, North American Cyanamid, Niagara Falls, Canada, for furnishing both samples of the ammonium nitrate. The list of the collaborators follows:

R. L. Willis and A. C. Wark, New Jersey Exp. Sta., New Brunswick
D. M. Salter, Ohio Department of Agriculture, Columbus
C. A. Butt and W. H. Banks, Int. Min. & Chem. Co., East Point, Georgia
L. J. Hardin and H. S. Johnson, Jr., Tenn. Agr. Exp. Sta., Knoxville
R. C. Koch and J. B. Hulsey, Swift & Co. Fert. Works, Hammond, Indiana
J. S. Kuzmeski and A. F. Spelman, Agr. Exp. Sta., Amherst, Massachusetts
Gordon Hart, Asst. State Chemist, Tallahassee, Florida
A. N. Lineweaver and C. T. McCloud, Royster Guano Co., Norfolk, Va.
C. V. Marshall, Dept. of Agriculture, Ottawa, Canada
Ralph D. Miller, Chief Chemist, Spencer Chem. Co., Pittsburg, Kansas
D. B. Bates, Smith-Douglass Co., Norfolk, Virginia
O. W. Ford and R. H. Hedrick, Agri. Exp. Sta., Lafayette, Indiana
C. O. Willits, In Charge Analytical Section, Eastern Reg. Lab., Philadelphia
P. R. Bidez, Alabama Dept. Agr., Auburn
W. A. Morgan, Ammonia Dept., Du Pont, Wilmington
J. W. MacKay, North American Cyanamid, Niagara Falls, Canada
M. M. Phillippe, Clemson Agr. College, Clemson, South Carolina
C. B. Jacobs, Davison Chem. Corp., Baltimore
H. R. Allen, Kentucky Agr. Exp. Sta., Lexington
C. Reynolds Clark, State Chemist, Atlanta, Georgia
Guy S. Mitchell, Chief Chemist, Lion Oil Co., El Dorado, Arkansas
John B. Smith, Chemist, Rhode Island Exp. Sta., Kingston
Philip McG. Shuey, Savannah, Georgia
C. O. Hurst and Archie G. McKee, Mississippi State Chem. Laboratory
Geo. C. Bollinger, Am. Agr. Chem. Co., Baltimore

The results on the three samples are given in Tables 1, 2, and 3.

PROCEDURE OF REPORTING

Results were not calculated to dry basis. The reasoning on this was that the variations on moisture determinations are too great. Where more

TABLE 1.—*Sample No. 1. Total nitrogen in pure ammonium nitrate*

ANALYST	DEVARDA METHOD	MgO DISTILLATION	NaOH DISTILLATION	FORMALDEHYDE TITRATION	MOISTURE
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
1	34.80	34.70	35.14		0.39
2	34.91	34.82	34.80		0.45
3	34.79	34.88	34.94	34.70	0.13
4	34.67	34.86	34.70	34.79	0.02
5	34.63	34.60	34.40	34.77	0.50
6	34.41	34.24	34.35	34.28	0.20
7	34.58	34.60	34.56	34.80	0.27
8	34.83	35.00	34.68		0.18
9	35.09	34.72	34.70	35.06	
10	34.46	34.85	34.70	34.94	0.00
11	34.82	34.76	34.79		
12	35.26	34.99	35.22	34.96	0.51
13	34.77	34.71	34.75		0.10
14	34.76	34.60	34.60		
15	34.89	34.98	35.02	34.94	0.05
16	34.34	34.50	34.62	34.38	
17	34.97	34.67	34.70		
18	34.48	34.50	34.56		0.18
19	34.83	34.88	34.92	34.89	0.28
20	30.40	34.40		34.78	3.31
21				34.96	0.10
22	34.57	34.48	34.72	34.67	0.26
23	34.89	34.84	34.78	34.66	0.21
24	34.90	34.85	34.90	34.92	0.13
25	34.81	34.87	34.90	34.86	0.05

than one bulb was used, merely the Corning No. 2020 was reported. This seems to be the most used bulb. One collaborator used the Davisson bulb, and two used the Iowa bulb, Corning No. 2040.

Owing to a late start this year, it seemed best to defer work on bulbs and traps. The Goessman trap is not necessary if one has a long condensing surface. The Davisson bulb may offer an advantage over the Corning No. 2020 in the Devarda procedure where there is a large blank. A paper describing a new connecting bulb[1] was presented at the meeting which may give us a basis for future work on this score.

DISCUSSION OF RESULTS

Sample No. 1:

This sample was of C. P. grade, and it theoretically contained 34.98% nitrogen (dry basis). If we glance at the results by the Devarda procedure, we see one result very low and two results slightly high. Over half of the other results approach this theoretical amount.

There were no absurd low or high results by the MgO distillation pro-

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[1] C. O. Willits, H. J. John, and L. R. Ross, *This Journal*, page 432.

Table 2.—*Sample No. 2. Total nitrogen in commercial ammonium nitrate*

ANALYST	DEVARDA METHOD	MgO DISTILLATION	NaOH DISTILLATION	FORMALDEHYDE TITRATION	MOISTURE
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
1	33.80	33.96	33.98		0.45
2	33.70	33.64	33.98		0.56
3	33.94	34.06	34.06	33.66	0.30
4	33.55	33.76	33.72	33.78	0.14
5	33.80	33.53	33.53	33.67	0.68
6	33.08	33.06	33.15	32.96	0.87
7	33.52	33.52	33.60	33.84	0.00
8	34.00	34.00	33.80		0.26
9	33.83	33.56	33.58	33.85	
10	33.58	33.97	33.84	34.01	0.00
11	33.87	33.77	33.69		
12	34.50	34.00	34.36	34.04	0.51
13	33.78	33.66	33.62		0.25
14	33.66	33.60	33.50		
15	33.86	33.80	33.78	34.00	0.17
16	33.32	33.48	33.43	33.56	
17	33.97	33.73	33.90		
18	33.80	33.88	33.76		0.28
19	33.80	33.76	33.84	33.87	0.41
20	29.90	33.00		33.86	2.73
21				34.09	0.03
22	33.58	33.76	33.44	33.64	0.43
23	33.62	33.71	33.66	33.42	0.40
24	33.88	33.81	33.89	33.90	0.29
25	33.84	33.81	33.89	33.75	0.22

cedure. There were two high results when using sodium hydroxide for distillation. The approach to the theoretical amount was about the same order as by the Devarda procedure. Of course, the ammoniacal nitrogen was multiplied by two.

It is regrettable that all did not try the formaldehyde titration method, since this probably offers the simplest possible method for the determination of nitrogen in ammonium nitrate. A cursory examination of the results submitted will show that this procedure seems to give equally as good results as the other methods. This procedure is described in a paper which was presented at the last meeting entitled "Method for Rapid Determination of Total Nitrogen in Ammonium Nitrate Fertilizer."[2]

Sample No. 2:

This sample theoretically contained 33.85% nitrogen. There was one very low result and two high results by the Devarda procedure. A majority of the analysts approached the theoretical amount, or were slightly over.

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[2] Ralph D. Miller, *This Journal*, page 373.

TABLE 3.—*Sample No. 3. Total nitrogen in mixed fertilizer (high chloride nitrate)*

ANALYST	KJELDAHL (2.27)	SHUEY METHOD	DYER HAMENCE	KJELDAHL (DOUBLE SALICYLIC)
	per cent	*per cent*	*per cent*	*per cent*
1	10.50	10.86		
2	10.20			
3	10.80			
4	10.52			
5	10.70			
6	10.55			
7	10.70	10.60		
9	10.03	9.58		
11	10.45			
12	10.78			
13	10.62			
14	10.83			
15	10.74	10.75		
17	10.58			
18	10.47			
19	10.45			
20	10.40			
22	10.68			
23	10.61	10.97		
24	10.75	10.74	10.86	10.86
25	10.82	10.84		

The nitrogen obtained by doubling the ammoniacal nitrogen produced by distillation with magnesium oxide and sodium hydroxide compares favorably with that obtained by the Devarda procedure. There are very few high results; but several failed to obtain the theoretical amount. Here, again, the formaldehyde titration shows up quite well.

Sample No. 3:

This sample was prepared in the Mississippi State Chemical Laboratory. The theoretical composition was 11.0–3.7–10.8. The nitrogen was from nitrate of soda and the potassium from potassium chloride.

No one obtained the theoretical amount of nitrogen by the Kjeldahl procedure (2.27). It is unfortunate that so few tried out the Shuey method, and the procedure suggested by Dyer and Hamence. One collaborator obtained 10.86 by this latter procedure, and he also obtained this amount when doubling the amount of salicylic in method 2.27. Another analyst obtained 10.97 by the Shuey method.

In Mississippi a 4% tolerance on the total value is allowed before a manufacturer is penalized. This type of material can certainly be determined within a 4% tolerance. However, many States are not so liberal; therefore, it is necessary to find a more exacting procedure.

OTHER METHODS

One analyst used the Ulsch method as revised by Street and obtained 34.84 and 33.85 per cent, respectively, on the two ammonium nitrate samples. This is probably best known as the Reduced Iron Method.

Another analyst used the Ferrous Sulfate-Zinc-Soda Method and obtained 34.92 and 33.76, respectively.

One analyst used Arnd alloy. He obtained 34.60, *vs.* his 34.80 with Devarda alloy, on sample No. 1; and he obtained 33.50, *vs.* 34.60 on sample No. 2. It has always been found in the Mississippi State Chemical Laboratory that the Arnd alloy gives lower results than the Devarda alloy.

Several collaborators reported results by the Devarda method on sample No. 3. These results were not given. They would apply to this particular mixture; however, not in general to this type of mixture.

COMMENTS BY COLLABORATORS

Analyst 1 believes that the Reduced Iron Method still has a place in our *Book of Methods.*

Analyst 7 gives a warning to the dangers of using selenium as a catalyst.

Analyst 9 prefers method **2.28** to **2.27.**

Analyst 12 thinks that either the magnesium oxide distallation, or the formaldehyde titration, offers a simpler approach to the determination of nitrogen in ammonium nitrate.

Analyst 13 gives analyses and a detailed discussion of bulbs. Out of deference to his paper which was presented to the Association, we shall omit the discussion here.

Analyst 15 brings out a good point. He suggests that the limitations should be worked out on method **2.27** for high nitrate-chloride mixtures.

Analyst 16 found no difference by Devarda procedure using a Goessman trap. He also points out the necessity of calcining the magnesium oxide, and the importance of using a small amount of sodium hydroxide for ammoniacal distillation of ammonium nitrate. He further warns us of the necessity of having conditions just right for the formaldehyde titration.

Analyst 19 prefers to allow his Devarda determinations to stand overnight before distilling.

Analyst 20 thinks the formaldehyde procedure is very simple.

Analyst 23 also thinks the formaldehyde procedure offers possibilities; however, he suggests using a smaller amount of sample.

CONCLUSIONS AND RECOMMENDATIONS*

All four methods, when properly handled, show possibilities for approaching the theoretical amount of total nitrogen in ammonium nitrate. The formaldehyde-titration procedure seems to be the simplest. There are apparently enough data available to set it up as a tentative method. However, more work and comparison are recommended before adopting it as official.

More work should be done on the nitrate-chloride mixtures. Working with dry samples, keeping our size of sample low, and increasing our salicylic, a nearer approach to the theoretical amount of nitrogen is possible.

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* For report of Subcommittee A and action by the Association, see *This Journal*, **31**, 42 (1948).

No report was given on magnesium and manganese, or on acid- and base-forming quality.

REPORT ON POTASH

By O. W. **Ford** (Purdue University Agricultural Experiment Station, Lafayette, Ind.), *Associate Referee*

In accordance with the recommendations of the Association that work on the details of the method for the determination of potash in commercial fertilizers be continued (*This Journal*, **30**, 228, 1947), referee work was conducted this year by collaboration. A copy of the proposed work and a set of fertilizer samples were sent to each chemist who had expressed a willingness to collaborate. This report summarizes the results of the fourteen chemists who found time to do the work and report to the Associate Referee.

Collaborative work on potash in fertilizers was directed to studies of the effect of increasing the concentrations of ethyl alcohol and formula 30 alcohol, and of saturating with potassium chloroplatinate the acid-alcohol from ethyl and formula 30 alcohols at different concentrations.

OUTLINE OF COLLABORATIVE WORK ON POTASH IN 1947

Suggestions to Collaborators

If collaborators cannot do all of the work outlined, they should complete individual sections. In reporting results, the report should include the kind of alcohol used, the amount used for each determination, and the concentration in terms of per cent by volume. Record all individual determinations as well as the average. Report by August 15, 1947. Contact the Associate Referee in case of doubt as to directions. Report all results by washing out the K_2PtCl_6 and weighing back. All should remember that the present official method calls for 80% ethyl alcohol by volume and when used the temperature of it should not exceed 30°C.

Samples selected for this work are a composite of several manufacturers' samples and should analyze approximately as follows: Sample number 4, analysis 0-9-27; sample number 5, analysis 3-9-18; sample number 6, analysis 0-0-55; sample number 8, analysis 4-12-8. All collaborators are urged to prepare a composite solution from each sample for all determinations.

PROCEDURE

Section I (80% ethyl alcohol and/or 80% formula 30 alcohol).—

A. Make six determinations of potash on samples 4, 5, 6, and 8 by the official method using 80% by volume ethyl alcohol and/or 80% formula 30 alcohol.

B. Repeat A, except that the acidified 80% ethyl alcohol or 80% formula 30 alcohol is previously saturated with K_2PtCl_6.

Section II (85% ethyl alcohol and/or 85% formula 30 alcohol).—

A. Repeat section I, A, except that 85% ethyl alcohol or 85% formula 30 alcohol be used.

- B. Repeat A, except that the acidified 85% ethyl alcohol or 85% compound 30 alcohol is saturated with K_2PtCl_6.

Section III (90% ethyl alcohol and/or 90% formula 30 alcohol).—

- A. Repeat section I, A, except that 90% alcohol ethyl or 90% formula 30 alcohol be used.
- B. Repeat A, except that the acidified 90% ethyl alcohol or 90% compound 30 alcohol is saturated with K_2PtCl_6.

Section IV (95% ethyl alcohol and/or 95% formula 30 alcohol).—

- A. Repeat section I, A, except that 95% ethyl alcohol or 95% formula 30 alcohol be used.
- B. Repeat A, except that the acidified 95% ethyl alcohol or 95% compound 30 alcohol saturated with K_2PtCl_6 be used.

COMMENTS ON RESULTS

The results of the chemists who collaborated on the potash work appear in Tables 1 and 2. Tables 3 and 4 show the average values obtained by the use of the two alcohols used alone and with the corresponding acid-alcohols saturated with K_2PtCl_6 at the varying concentrations.

From the list of collaborators in Tables 1 and 2 it will be noted that the commercial chemists all used formula 30 alcohol and the control chemists used ethyl alcohol for this work. Only one chemist, collaborator 14, found time to use both alcohols in the studies. This was to be expected from past years' reports, as the commercial chemist cannot obtain ethyl alcohol tax-free for this work and because of the difference in cost, they usually use formula 30. Many commercial chemists have been using formula 30 for years even though the method was developed using ethyl alcohol. This work could be justified because a comparison of the two alcohols is needed.

1. With 80% formula 30 alcohol in 26 of 28 cases more potash was obtained when the acid-alcohol was saturated with K_2PtCl_6 (Table 2). The average increase in potash for the four samples was 0.18% with differences ranging from 0.09% to 0.27%. In most cases the greater the K_2O content the greater the increase.

2. With 85% formula 30 alcohol in 23 of 24 cases more potash was obtained by saturation of the acid-alcohol with K_2PtCl_6. The average increase was 0.12% and differences ranged from 0.08% to 0.20%.

3. With 90% formula 30 alcohol, in 18 of 24 cases more potash was obtained by saturation of the acid-alcohol with K_2PtCl_6. The average increase was only 0.06% with differences ranging from 0.02% to 0.11%.

4. With 95% formula 30 alcohol, in 32 of 36 cases more potash was obtained by saturation of the acid-alcohol with K_2PtCl_6. The average increase was 0.05% and differences ranged from 0.04% to 0.06%. As the concentration of the alcohol was increased, the amount of increase of potash by saturation of the acid-alcohol became less, which indicates that saturation of the acid-alcohol has a great effect on the amount of potash obtained at the lower concentrations of alcohol. This becomes less as the alcohol concentration is increased due to the smaller solubility of K_2PtCl_6 in the more concentrated alcohol.

TABLE 1.—*Effect of concentration of ethyl alcohol and acid-alcohol and saturation of the acid-alcohol with K_2PtCl_6 on the determination of potash in fertilizers*

ANALYST NO.	NO. OF ANALYSES	80% BY VOLUME		85% BY VOLUME		90% BY VOLUME		95% BY VOLUME	
		NOT SATURATED	SATURATED WITH K_2PtCl_6	NOT SATURATED	SATURATED WITH K_2PtCl_6	NOT SATURATED	SATURATED WITH K_2PtCl_6	NOT SATURATED	SATURATED WITH K_2PtCl_6
SAMPLE #4 0-9-27 ANALYSIS									
6	3	26.48	26.79	26.59	27.03	26.80	27.04	26.98	27.04
7	6	26.94	26.95	26.85	27.15	26.85	27.06	27.20	27.13
11	3	26.69	26.74			26.76	26.90		
12	6	26.63	27.15	26.76	27.25	26.97	27.26	27.15	27.26
14	6	27.27	27.64	28.00	28.20	27.88	28.15	28.23	28.32
Average		26.82	27.05	27.05	27.40	27.05	27.28	27.39	27.44
Av. increase due to saturation			+0.23		+0.35		+0.23		+0.05
SAMPLE #5 3-9-18 ANALYSIS									
6	3	18.27	18.69	18.37	18.85	18.55	18.87	18.65	18.93
7	6	18.26	18.45	18.40	18.58	18.46	18.55	18.16	17.90
11	3	18.70	18.82			18.80	18.92		
12	6	18.50	18.81	18.79	18.83	18.87	18.87	18.88	18.91
14	6	18.51	18.82	18.70	18.70	18.64	18.64	18.80	18.94
Average		18.45	18.72	18.57	18.74	18.66	18.77	18.62	18.67
Av. increase due to saturation			+0.27		+0.17		+0.11		+0.05

TABLE 1.—(*continued*)

ANALYST NO.	NO. OF ANALYSES	80% BY VOLUME		85% BY VOLUME		90% BY VOLUME		95% BY VOLUME	
		NOT SATURATED	SATURATED WITH K_2PtCl_6	NOT SATURATED	SATURATED WITH K_2PtCl_6	NOT SATURATED	SATURATED WITH K_2PtCl_6	NOT SATURATED	SATURATED WITH K_2PtCl_6
SAMPLE #6 0-0-55 ANALYSIS									
6	3	58.99	59.04	59.09	59.53	59.11	59.55	59.24	59.58
7	6	59.17	59.58	60.07	60.24	59.10	59.14	59.48	59.66
11	3	59.03	59.07			59.29	59.31		
12	6	57.02	57.52	57.11	57.55	57.32	57.59	57.45	57.53
14	6	57.70	58.40	57.80	58.38	57.84	58.37	57.75	57.87
Average		58.38	58.72	58.52	58.92	58.53	58.79	58.48	58.66
Av. increase due to saturation			+0.34		+0.40		+0.26		+0.18
SAMPLE #8 4-12-8 ANALYSIS									
6	3	7.81	8.03	7.85	8.12	7.84	8.17	7.88	8.19
7	6	8.06	8.13	8.21	8.38	8.38	8.51	8.53	8.61
11	3	7.74	7.82			7.84	7.86		
12	6	7.86	8.02	7.95	8.02	8.03	8.03	8.03	8.03
14	6	8.35	8.76	8.54	8.86	8.52	8.65	8.45	8.57
Average		7.96	8.15	8.14	8.35	8.12	8.24	8.22	8.35
Av. increase due to saturation			+0.19		+0.21		+0.12		+0.13

TABLE 2.—*Effect of concentration of formula 30 alcohol and acid-alcohol and saturation of the acid-alcohol with K_2PtCl_6 on the determination of potash in fertilizers*

ANALYST NO.	NO. OF ANALYSIS	80% BY VOLUME		85% BY VOLUME		90% BY VOLUME		95% BY VOLUME	
		NOT SATURATED	SATURATED WITH K_2PtCl_6	NOT SATURATED	SATURATED WITH K_2PtCl_6	NOT SATURATED	SATURATED WITH K_2PtCl_6	NOT SATURATED	SATURATED WITH K_2PtCl_6
SAMPLE #4 0-9-27 ANALYSIS									
1	6	26.92	27.00	26.94	26.93	27.14	27.07	27.09	27.08
2	6	26.59	26.83					27.00	27.09
3	6	26.64	26.68	26.62	26.76	26.70	26.71	26.73	26.75
4	6	26.71	26.92	27.00	27.11	26.96	27.12	27.05	27.16
5	6	26.88	26.89	26.89	26.89	26.92	26.92	26.94	26.96
8	6							26.89	26.97
9	6	26.55	27.12	26.89	27.06	26.91	27.06	27.00	27.09
10	6							26.34	26.46
14	6	27.75	27.89	27.88	28.03	27.84	27.88	27.96	27.95
Average		26.86	21.07	27.03	27.12	27.08	27.13	27.00	27.06
Av. increase due to saturation			+0.21		+0.09		+0.05		+0.06
SAMPLE #5 3-9-18 ANALYSIS									
1	6	18.38	18.39	18.55	18.55	18.63	18.63	18.62	18.66
2	6	18.41	18.60					18.64	18.66
3	6	18.40	18.51	18.49	18.49	18.51	18.53	18.57	18.59
4	6	18.47	18.59	18.64	18.69	18.62	18.66	18.70	18.74
5	6	18.56	18.59	18.56	18.65	18.70	18.73	18.79	18.79
8	6							18.88	18.97
9	6	18.59	18.88	18.67	18.95	18.76	18.94	18.74	18.79
10	6							18.16	18.31
14	6	17.69	17.93	17.89	17.95	17.82	17.96	17.82	17.87
Average		18.35	18.49	18.47	18.55	18.34	18.41	18.55	18.60
Av. increase due to saturation			+0.14		+0.08		+0.07		+0.05

TABLE 2.—(*continued*)

SAMPLE #6 0-0-55 ANALYSIS

ANALYST NO.	NO. OF ANALYSES	80% BY VOLUME		85% BY VOLUME		90% BY VOLUME		95% BY VOLUME	
		NOT SATURATED	SATURATED WITH K_2PtCl_6	NOT SATURATED	SATURATED WITH K_2PtCl_6	NOT SATURATED	SATURATED WITH K_2PtCl_6	NOT SATURATED	SATURATED WITH K_2PtCl_6
1	6	58.14	58.32	58.26	58.39	58.54	58.62	58.60	58.65
2	6	56.92	56.94					57.40	57.50
3	6	59.31	59.20	59.22	59.36	59.39	59.35	59.49	59.56
4	6	57.13	57.40	57.33	57.42	57.26	57.41	57.49	57.53
5	6	57.67	57.90	57.83	57.80	57.79	57.80	57.78	57.79
8	6							57.00	57.06
9	6	58.96	59.92	59.13	59.82	59.18	59.54	59.56	59.59
10	6							58.90	59.12
14	6	56.95	57.30	57.19	57.35	57.13	57.22	57.28	57.28
Average		57.87	58.14	58.16	58.36	58.21	58.32	57.05	57.11
Av. increase due to saturation			+0.27		+0.20		+0.11		+0.06
SAMPLE #8 4-12-18 ANALYSIS									
1	6	8.28	8.30	8.30	8.36	8.33	8.32	8.32	8.37
2	6	7.80	7.85					8.03	8.08
3	6	7.97	7.96	7.96	8.00	8.10	8.07	8.12	8.11
4	6	7.78	7.99	7.86	7.93	7.90	7.94	7.93	7.96
5	6	7.88	7.93	7.95	8.09	8.08	8.07	8.06	8.16
8	6							8.11	8.16
9	6	7.94	8.24	8.00	8.29	8.06	8.25	8.08	8.15
10	6							7.77	7.86
14	6	8.55	8.60	8.54	8.61	8.73	8.62	9.33	9.24
Average		8.03	8.12	8.10	8.21	8.20	8.22	8.26	8.30
Av. increase due to saturation			+0.09		+0.11		+0.22		+0.04

TABLE 3.—*Ethyl alcohol and acid-alcohol saturated with K_2PtCl_6 vs. formula 30 alcohol and acid-alcohol saturated with K_2PtCl_6 in the determination of potash in fertilizer at 80, 85, 90, and 95% by volume concentrations*

ALCOHOL CONCENTRATION	TYPE OF ALCOHOL	SAMPLE NUMBER			
		4	5	6	8
80	Ethyl	27.10	18.72	59.68	8.15
80	Formula 30	27.07	18.49	58.14	8.12
85	Ethyl	27.40	18.63	58.92	8.35
85	Formula 30	27.12	18.55	58.36	8.21
90	Ethyl	27.37	18.73	58.75	8.34
90	Formula 30	27.13	18.41	58.32	8.22
95	Ethyl	27.32	18.72	57.24	8.25
95	Formula 30	27.06	18.60	57.11	8.30

5. Slightly larger increases of potash were obtained by the saturation of the acid-alcohols of ethyl alcohol at the different concentrations than with formula 30 alcohol.

6. In most cases both formula 30 and ethyl alcohol gave higher potash values with an increase in alcohol concentration (Tables 3 and 4). The greatest discrepancy was with sample #6. This was a mixture of potash salts and evidently subject to segregation so that if all the determinations were not made from a composite solution as indicated in the outline of the work, erratic results would be obtained. This is about the only way one could account for the great variation in potash values reported by the collaborators for this sample. Here again, however, most collaborators ob-

TABLE 4.—*Ethyl alcohol and acid-alcohol not saturated with K_2PtCl_6 vs. compound 30 alcohol and acid-alcohol not saturated with K_2PtCl_6 in the determination of potash in fertilizer at 80, 85, 90, and 95% by volume concentration*

ALCOHOL CONCENTRATION	TYPE OF ALCOHOL	SAMPLE NUMBER			
		4	5	6	8
80	Ethyl	26.82	18.45	59.32	7.96
80	Formula 30	26.86	18.35	57.87	8.03
85	Ethyl	27.05	18.47	58.52	8.14
85	Formula 30	27.03	18.47	58.16	8.10
90	Ethyl	27.12	18.63	58.44	8.19
90	Formula 30	27.08	18.34	58.21	8.20
95	Ethyl	27.06	18.66	57.09	8.15
95	Formula 30	27.00	18.55	57.05	8.26

tained more potash both by saturation of the acid-alcohol and by use of an increased alcohol concentration.

7. In general about 0.1% more potash was obtained by using ethyl alcohol than by using formula 30 alcohol. This was true whether the acid-alcohol was saturated with K_2PtCl_6 or used without saturation.

Since approximately the same amount of potash can be obtained by saturation of acid-alcohol or by increasing the concentration of the alcohol, it would seem that the choice of conditions would most logically depend on the ease and convenience of laboratory manipulation. In most laboratories the use of more concentrated alcohol is more convenient than the saturation of the acid-alcohol with K_2PtCl_6.

TABLE 5.—*Kind of alcohol and part of the equipment used in 1947 collaborative potash work*

ANALYST NO.	KIND OF ALCOHOL	PER CENT ALCOHOL BY VOLUME	VOLUME OF ALCOHOL	KIND OF DISH CAPACITY	TYPE OF FILTER, TEMPERATURE OF ALCOHOL
1	Formula 30	80, 85, 90, 95	150 ml	Platinum 75 ml	Asbestos Gooch 20–21° C.
2	Formula 30	80, 95	125–150 ml		
3	Formula 30	80, 85, 90, 95			
4	Formula 30	80, 85, 90, 95			
5	Formula 30	80, 85, 90, 95	150 ml	Silica and porcelain 90 ml	20–25° C.
6	Ethyl	80, 85, 90, 95			
7	Ethyl	80, 85, 90, 95			
8	Formula 30	95			
9	Formula 30	80, 85, 90, 95	95–105 ml		
10	Formula 30	95	115–120 ml		
11	Ethyl	80, 90			
12	Ethyl	80, 85, 90, 95			
13	Ethyl	80, 85, 90, 95			
14	Ethyl	80, 85, 90, 95	125 ml	Platinum 125 ml	Sintered Pyrex M. 20–25° C.
	Formula 30	80, 85, 90, 95	125 ml	Platinum 125 ml	Sintered Pyrex M. 20–25° C.

Ewan, Ford, and Schall (1) and Schall and Ford (2) have shown that increases in the potash values obtained by increasing the alcohol concentration and by saturation of the acid-alcohol with K_2PtCl_6 are true potash values within the limits of experimental error. Therefore, the Associate Referee recommends that 95% ethyl alcohol and acid-alcohol or 95% formula 30 alcohol and acid-alcohol without saturation of the acid-alcohol with K_2PtCl_6 replace 80% ethyl alcohol and acid-alcohol for the determination of potash in commercial fertilizers. The Associate Referee (from a Commercial Solvent Corporation pamphlet) understands that formula 30 is a mixture of 100 gallons of ethyl alcohol and 10 gallons of pure methyl alcohol.

LIST OF COLLABORATORS

(1) Austin, W. R., and Buford, Madelane, Armour Fertilizer Works, Nashville, Tenn.
(2) Bates, D. B., Smith-Douglass Company, Inc., Norfolk, Va.
(3) Batton, H. C., Swift and Company, Norfolk, Va.
(4) Bollinger, G. C., Trimble, C. E., Charlton, R. C., The American Agricultural Chemical Company, New York City, N. Y.
(5) Byers, C. R., and Vaugh, H., Armour Fertilizer Works, Carteret, N. J.
(6) Etheredge, M. P., Mississippi State Chemical Laboratory, State College, Miss.
(7) Fudge, J. F., Ogier, T. L., and co-workers, Agricultural and Mechanical College of Texas, College Station, Tex.
(8) Lineweaver, A. N., F. S. Royster Guano Company, Norfolk, Va.
(9) Powell, R. O., and Moxon, H. L., Virginia-Carolina Chemical Corporation, Richmond, Va.
(10) Shuey, P. Mc., Shuey & Company, Savannah, Ga.
(11) Smith, C. Tyson, and Kuzmeski, J. W., Agricultural Experiment Station, Amherst, Mass.
(12) Smith, R. M., Agricultural Department, Chemical Division, Tallahassee, Fla.
(13) Willis, R. L., Rutgers University, Agricultural Experiment Station, New Brunswick, N. J.
(14) Schall, E. D., Purdue University, Agricultural Experiment Station, Lafayette, Ind.

COMMENTS OF COLLABORATORS

(1) We favor the control of temperatures even though it means some additional trouble and work, since judging from previous years work on this collaborative work, and regular work too, that weaker alcohols and normal summer temperatures found in many laboratories do give high solubility of potash in a great many instances coming under our observation.

We prefer the selection of 90% or 85% alcohol as giving on the average the more satisfactory results, as compared with 80% which we think is too low for average routine work, while at times the 95% strength does not give as clean a precipitate as the preferred strengths do give.

(2) We use a muffle furnace for burning down our potash and found that by increasing our time in the furnace the back weight, after washing out the K_2PtCl_6, was kept at a minimum. We feel that the back weight found is due to the incomplete burning off and subsequent burning of the potash.

There was a definite amount of residue.

(5) Use factor weight 1.938 grams in 250 and 25 ml aliquots equivalent to .1938 grams sample instead of 2.5 grams in 250 ml.

(10) From .09% to 0.22% K_2O increase of potash obtained by the saturation of the acidified formula 30 alcohol.

(12) In saturating the alcohols this year, I found a slight reduction or reducing effect on the potassium-chloro-platinate as evidenced by the precipitate turning brown and the solution taking on a very deep, brownish color which effect was more pronounced with the weaker alcohols. This is the first time that I have noticed this effect on saturating alcohols and I am wondering if the alcohols we are getting today are different in any way than in former years. However, when the alcohol is filtered and used in the tests, it seems to make no apparent difference. I much favor the use

of 95% alcohol when possible. Where this is not possible, it seems to me that alcohol of better than 80% should be recommended, even though saturated.

(14) Favor the use of more concentrated alcohol than the present 80% and for ease and accuracy of laboratory manipulation omit the saturation of the acid-alcohol with K_2PtCl_6.

ACKNOWLEDGMENT

The thanks of the Associate Referee are extended to F. W. Quackenbush and E. D. Schall of the Agricultural Chemistry Department, Purdue University, for valuable suggestions and criticisms in the development of the investigations covered by this report. In addition, thanks are extended to the other collaborators for their cooperation.

RECOMMENDATIONS*

It is recommended—

(1) That the 80% alcohol used in the determination of potash in fertilizers be changed to 95% ethyl alcohol or 95% formula 30 alcohol.

(2) That section **2.40 (d)** of the *Methods of Analysis* be changed to read: **(d)** *Acid-alcohol.*—Mix 200 ml of 95% ethyl alcohol, or 95% formula 30 alcohol, with 20 ml of concentrated HCl and cool to room temperature (official, first action).

(3) That all references to 80% alcohol in section **2.42 (a)** of the *Methods of Analysis* (1945) be changed to 95% ethyl alcohol or 95% formula 30 alcohol (official, first action).

LITERATURE CITED

(1) Ewan, M. A., Ford, O. W., and Schall, E. D., *Anal. Chem.* (in press).
(2) Schall, E. D., and Ford, O. W., *This Journal*, page 397.

No reports were given on sulfur, copper and zinc, or boron in fertilizers. For report on boron under Plants, see page 284.

REPORT ON CEREAL FOODS

By V. E. Munsey (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

The Associate Referee on soy bean flour has submitted no report. He has, however, kept in contact with the Committee on Seed and Meal Analyses of the American Oil Chemists Society and submitted their report on the analysis of soy bean flour. This committee has investigated the methods of analysis for soy bean flours for moisture and volatile matter, oil, ash, protein, and crude fiber. Its findings are published in *J. Am. Oil Chem. Soc.* **24**, 303 (1947). Their study covers the three types of soy flour, *i.e.*, high fat, low fat, and defatted flours. The method for moisture specifies 5 g at 130°C. for 2 hours, in a forced draft oven, while the present tentative method dries 2 g at 130° for 1 hour in an air oven. This additional hour at 130° approaches very closely the conditions of the official air oven method for feeds (2 hours at 135°) which includes soy bean meal. The method for oil is essentially the same as the present tentative method, with the exception that the extraction period is specified for 5 hours and weight of sample at ca 2 g for full fat flour and 5 g for low or defatted flour. The method for ash is the same as the official method for feed. This requires ignition of 2 g sample in previously heated muffle furnace at 600°C. and maintained at this temperature (± 15°C.) for 2 hours with automatic pyrometer control, whereas the present tentative method ignites at 550°C. until a gray ash results. Crude fiber is the same as the present tentative method. The nitrogen varies from the present tentative method in that the amount of K_2SO_4 or Na_2SO_4 is specified at 10 g and the catalyst limited to HgO or its equivalent in metallic Hg and no use of $Na_2S_2O_3$ solution. The Associate Referee emphasizes the advantage of the adoption of the same methods by the A.O.C.S. and the A.O.A.C.

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* For report of Subcommittee A and the action of the Association, see *This Journal*, **31**, 42 (1948).

RECOMMENDATIONS*

It is recommended—

(1) That both procedures proposed by the Associate Referee for the determination of phosphorus in cereals and cereal products be adopted official (first action) and the study be continued.

(2) That the dry ashing method for iron **20.9–20.12**, inclusive, be made official (final action) for enriched macaroni products, degerminated, bolted and whole corn meals, and that the study be discontinued.

(3) That the wet ashing method for iron (*This Journal*, **30**, 71, 1947) be made official (final action) and that study be discontinued.

(4) That the studies on determination of starch in raw and cooked cereals be continued.

(5) That the tentative method for the determination of fat acidity in grain, flour, corn meal, and whole wheat flour (**20.18–20.21**, inclusive) be further studied and also that the relationship of acidity to unsoundness be studied.

(6) That the Associate Referee continue his work reported this year on the application of the method for reducing and non-reducing sugars in flour **20.28–20.30**, inclusive, to the determination of sugars in bread and other bakery products, with special consideration to the article on this subject published by R. M. Sandstedt and G. C. Fleming (*This Journal*, **30**, 550–52).

(7) That the tentative method for benzoyl peroxide in flour, **20.53**, be continued for rye flour, and that the method proposed by the Associate Referee replace it for wheat flour.

(8) That work be continued on methods for determination of available CO_2 in self-rising flour containing added $CaCO_3$.

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* For report of Subcommittee D and action of the Association, see *This Journal*, **31**, 58 (1948).

(9) That the method for the determination of lactose in bread (*This Journal*, **24**, 630) be further studied.

(10) That the determination of milk fat in bread, **20.86**, be further studied.

(11) That the methods for the determination of proteolytic activity of flour be continued.

(12) **(a)** That the method for moisture in soy flour, **20.77**, be changed to read—"Moisture—*see* **20.2** or **20.4**, with the exception that a 5 g sample be dried 130° for 2 hours." **(b)** That the method for ash, **20.78**, be changed to read—"Ash—*see* **27.9**." **(c)** That the method for nitrogen, **20.79**, be changed to read—"Proceed as directed under **2.26**, using 10 g K_2SO_4 or Na_2SO_4 and 0.7 g of HgO or its equivalent in Hg with the additional option of using sodium alizarin sulfonate." **(d)** That the method for oil, **20.82**, be changed to read "See **31.67** except that ca 2 g full-fat flour or 5 g low or defatted soy flour be extracted for 5 hours" and that the study be continued on these methods.

(13) That studies be made on the detection and determination of soy bean flour in cereal products.

(14) That the method proposed by the Associate Referee for determination of the amount of added inorganic material in phosphated and self-rising flour be further studied.

(15) That the method referred to in *This Journal*, **25**, 83–84, for the determination of unsaponifiable matter and sterols in noodles be studied to determine their applicability to other foods containing eggs.

(16) That studies of methods for the determination of albumin in noodles and other farinaceous egg-containing products be continued.

(17) That the method for the determination of total solids, **20.84 (b)**, for raisin bread and bread containing raisins and fruits adopted as official first action *This Journal*, **30**, 72 (1947) be adopted as official (final action), and that study on the determination of moisture, fat, crude fiber, ash, and protein in bakery products be continued.

(18) That the study on the determination of moisture in all flour-like products containing sodium bicarbonate as one of its constitutents be continued.

(19) That the study on the determination of bromates in flour be continued along the lines suggested in this year's report of the Associate Referee.

(20) That the study on the determination of apparent viscosity measurements of flour be discontinued.

No reports were given on starch in raw and cooked cereals, or on fat acidity in grain, flour, corn meal, and whole wheat flour.

REPORT ON BENZOIC ACID IN FLOUR

By V. E. MUNSEY (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

The present tentative method for the determination of benzoic acid in flour (*Methods of Analysis*, sec. **20.53**, p. 253) is long, inconvenient, and variable recoveries of benzoic acid result by this steam distillation procedure. A direct extraction of flour acidified with various amounts each of HCl and H_2SO_4 indicate organic solvents can be used. The same flour bleached with benzoyl peroxide gave the following recoveries on extraction of the HCl acidified flour with four solvents, *i.e.*, petroleum ether 20 per cent, benzene 31 per cent, ether 88 per cent, and isopropyl ether 89 per cent. Results on known amounts of benzoic acid added to flour showed a 94, 94, and 103 per cent recovery with ether, and 93 per cent with isopropyl ether. However, the recoveries for added known amounts of benzoyl peroxide were variable, depending on the degree of conversion of the peroxide to benzoic acid by the flour. Reducing substances were added to the flour before acidification with HCl to effect conversion of any benzoyl peroxide not completely changed to benzoic acid by the flour. A freshly prepared mixture of flour and a benzoyl peroxide bleaching agent was analyzed by adding ferrum reductum, hydroquinone, hydroxylamine, sodium hydrosulfite, and stannous chloride in approximately equivalent amounts to the flour before acidification with HCl. The hydroquinone, hydroxylamine, and sodium hydrosulfite gave the same results as the control, indicating no reduction of the peroxide. The stannous chloride gave a 63 per cent conversion but caused interference in the procedure. Ferrum reductum gave 100 per cent recovery and appeared to be an ideal reducing agent without any interference. A few commercial flours bleached with benzoyl peroxide gave 92–95 per cent as much benzoic acid without treatment with ferrum reductum. The small amount of interfering pigments extracted from flour was reduced somewhat by the use of carbon (Nuchar W) without loss of benzoic acid. Benzoic acid in acetone added to ether and analyzed showed 101 and 96 per cent recovery without carbon, and 97 and 95 per cent with addition of carbon. The satisfactory application of the above principles led to the submission of a tentative procedure to 12 collaborators. The method in detail is given in *This Journal*, **31**, 80 (1948), under "Changes in Methods of Analysis."

Three samples of flour were sent to each collaborator. Sample No. 1 had no added benzoyl peroxide. Sample Nos. 2 and 3 had about 8.7 and 17.4 mg of benzoic acid per 100 g of flour, respectively, based on calculation from an analysis of the benzoyl peroxide bleaching agent. The collaborators were asked to report both the qualitative and quantitative results. The results from eight collaborators are given in Table 1.

TABLE 1.—*Benzoic acid in flour*

COLLABORATOR	SAMPLE NO. 1	SAMPLE NO. 2	SAMPLE NO. 3
	p.p.m.	*p.p.m.*	*p.p.m.*
1	—	6.3	13.5
2	5.6	11.0	13.1
3	—	10.6	10.4
4	2	5	10
5	negative	positive	positive
6	0.1	8	10
7	0	11.2	20.0
		10.4	19.2
8	0.5	7.6	15.6
		8.2	15.8

In general these results do not indicate satisfactory quantitative measurements. They do indicate the degree of bleaching in accordance with the amount added and definitely indicate a flour with normal commercial bleach with benzoyl peroxide. Sample 3 contained benzoyl peroxide in the amount commonly used commercially, while sample 2 is considerably below usual practice. The qualitative results were correct except for one collaborator who thought the faint color in the untreated sample No. 1 might represent a little benzoic acid. Collaborator 4 reports 100 per cent recovery of known added amounts of benzoic acid to flour by this method. While the quantitative collaborative results did not measure up to that expected by the Associate Referee, the qualitative results indicate the method is satisfactory for the detection of benzoyl peroxide-treated flour. Further work should be done on the quantitative measurement.

The Associate Referee wishes to gratefully acknowledge the assistance of the following collaborators:

R. L. Gray, Lucidol Division, Novadel-Agene Corp., Buffalo, N. Y.
K. L. Fortmann, Novadel-Agene Corp., Newark, N. J.
W. L. Rainey, Commander Larabee Milling Co., Minneapolis, Minn.
J. J. Winston, Jacobs Products Laboratory, Inc., New York, N. Y.
R. C. Koehn, General Mills, Inc., Minneapolis, Minn.
F. J. McNall and M. A. Braun, of Food and Drug Administration, Dept. of Agriculture.

It is recommended* that the tentative method for benzoyl peroxide in flour, **20.53**, be continued for rye flour and the title changed to "Benzoic Acid in Rye Flour"; and that the proposed method replace it for wheat flour.

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* For report of Subcommittee D and action of the Association, see *This Journal*, 31, 58 (1948).

REPORT ON SUGAR DETERMINATIONS IN BAKED PRODUCTS

By NILES H. WALKER (National Biscuit Company, New York, N. Y.), *Associate Referee**

The common sugars which occur in bakery products are dextrose, levulose, lactose, maltose, and sucrose. It is difficult to determine actual percentages of the various sugars in mixtures which often occur. Approximations can be made by special methods. In lieu of identity, it usually suffices to determine the quantities of reducing sugars, and of non-reducing sugars which hydrolyze and produce reducing sugars. Since the greater amount of reducing sugars in most bakery products is hexoses, *i.e.*, invert sugar from hydrolyzed sucrose, and dextrose and levulose from syrups, honey, and fruits, it seems reasonable to calculate and report reducing sugars as invert sugar unless identities are definitely known or established by special determinations. Practically all of the non-reducing sugar content, which is readily inverted to produce invert sugar by the usual procedures, is sucrose. It may be calculated as such without introducing any appreciable quantitative error.

The tentative method for the determination of sugars in baked products, **20.103** (1)[1] requires the extraction of sugars with boiling 50 per cent alcohol, evaporation of alcohol from an aliquot of the extract, taking up with water, clarification with neutral lead acetate, and deleading the solution before determinations can be carried out. This requires appreciable time and in many cases clarification is unsatisfactory. Water extraction with acid tungstate clarification has been found satisfactory for the determination of the maltose value (2, 3, 4) of flour. Methods for the determination of sucrose (5) after inversion by the acid added for clarifica-

TABLE 1.—*Per cent sugars**—*Method* **20.95, 20.103**

SAMPLE	SUGARS		
	REDUCING AS INVERT	SUCROSE	TOTAL
Wheat flour (12% moisture basis)	.20	1.08	1.28
Bread (38% moisture basis)	2.28	.24	2.52
Soda crackers	.97	.37	1.34
No. 1 (Sweet cracker)	4.31	18.39	22.70
No. 2 (Sweet cracker)	4.74	18.17	22.91
No. 3 (Sweet cracker)	7.18	15.65	22.83
No. 4 Honey Graham cracker	6.75	15.28	22.03
No. 5 Graham cracker	3.81	16.37	20.18

* All results in duplicate.

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* Present address, Port Chester, N. Y., Arnold Bakers, Inc.

[1] NOTE.—References in bold face figures are all to sections in *Methods of Analysis*, 6th Ed., 1945.

tion, and the determination of the original reducing sugars, (6) by the ferricyanide procedure on water extractions from flour after clarification with acid tungstate, have also proved satisfactory.

In accordance with the recommendation of the Association, a study was started on the application of the method for determining reducing and non-reducing sugars in flour, **20.29**, to the determination of sugars in other baked products. Sugar determinations were made on wheat flour, white bread, and six varieties of crackers by the tentative method **20.95, 20.103** given for the determination of sugars in baked products, and adaptations of the official method, **20.28–20.30**, for the determination of reducing and non-reducing sugars in flour.

Table 1 gives results obtained by tentative method **20.95, 20.103.** Sugars were determined by the Munson-Walker general method. Cuprous oxide was weighed and the invert sugar equivalent taken from Table **44.11.**

Table 2 gives results obtained by adaptations of the official method **20.28–20.30** for the determination of reducing and non-reducing sugars in flour. Reagents used were the same as listed in **20.28.** Reducing sugars were calculated as invert. The weight of sample used was limited by the amount of sugar it contained. Adjustments were made to meet volume

TABLE 2.—*Per cent sugars*—Method* 20.28–20.30

SAMPLE		SUGARS		
		REDUCING AS INVERT	SUCROSE	TOTAL
Wheat flour (12% moisture basis) 5.675 gm.		.12	1.60	1.72
Bread (38% moisture basis) 2.8375 gm. (6% moisture basis)		2.05	.25	2.30
Soda cracker 5.675 gm.		.93	.48	1.41
No. 1 (Sweet cracker)	.7094 gm.	3.94	19.60	23.54
No. 2 (Sweet cracker)	.7094 gm.	4.36	19.20	23.56
No. 3 (Sweet cracker)	.7094 gm.	6.96	16.76	23.72
No. 4 Honey Graham cracker	.7094 gm.	5.90	17.12	23.02
No. 5 Graham cracker	.7094 gm.	3.80	17.36	21.16
Wheat flour (12% moisture basis) 5 gm. in 100 ml. vol.		.12	1.62	1.74
Bread (38% moisture basis) 5 gm. (6% moisture basis) in 100 ml. vol.		2.09	.25	2.34
Soda cracker 5 gm. in 100 ml. vol.		.99	.41	1.40
No. 1 (Sweet cracker) 1.25 gm. in 100 ml. vol.		3.82	20.08	23.90
No. 2 (Sweet cracker) 1.25 gm. in 100 ml. vol.		4.27	19.47	23.74
No. 3 (Sweet cracker) 1.25 gm. in 100 ml. vol.		6.93	16.25	23.18
No. 4 Honey Graham cracker 1.25 gm. in 100 ml. vol.		5.90	17.00	22.90
No. 5 Graham cracker 1.25 gm. in 100 ml. vol.		3.48	16.92	20.40

* All results in duplicate.

requirements where the amount of alcohol required to wet the charge was reduced. Charges made to volume and extracted in volumetric flasks were treated as nearly as possible as directed for the wetting and extraction of charges in Erlenmeyer flasks. Corrections were made for space occupied by insoluble solids in volumetric flasks.

Table 3 gives results of calculated sugar contents of three varieties made from weight and analysis of ingredients.

TABLE 3.—*Per cent sugars calculated from weight and analyses of ingredients*

SAMPLE	SUGARS		
	REDUCING AS INVERT	SUCROSE	TOTAL
No. 1 (Sweet cracker)	4.20	18.30	22.50
No. 2 (Sweet cracker)	4.18	18.50	22.68
No. 3 (Sweet cracker)	7.05	15.80	22.85

DISCUSSION

In general, results obtained by method **20.28–20.30**, seem slightly high especially for the crackers with the higher sugar contents.

Clarifications of extracts from most of the crackers were not very satisfactory with either neutral lead acetate or acid tungstate.

Although results obtained on extracts made as directed in **20.29** agreed within reasonable tolerances with those made in volumetric flasks (Table 2), it seems simpler and more practical to make the extractions in volumetric flasks where volumes can be more accurately measured.

Acknowledgment is made to Mr. E. K. Spotts (National Biscuit Company, N. Y.), who collaborated with the author in this work.

It is recommended* that further work be conducted in an effort to adapt method **20.28, 20.29, 20.30** to the determination of sugars in baked products.

LITERATURE CITED

(1) *Methods of Analysis, A.O.A.C.*, Sixth Edition, 1945.
(2) RUMSEY, American Institute of Baking Bulletin 8 (1922).
(3) BLISH, M. J., *This Journal*, **16**, 497 (1933).
(4) BLISH and SANDSTEDT, *Cereal Chemistry*, **10**, 189 (1933).
(5) SANDSTEDT, R. M. *Cereal Chemistry*, **14**, 767 (1937).
(6) SANDSTEDT, R. M. *This Journal*, **22**, 535 (1939).

No report was given on carbon dioxide in self-rising flour.

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* For report of Subcommittee D and action of the Association, see *This Journal*, **31**, 58 (1948).

REPORT ON PHOSPHATED FLOUR

By Frank H. Collins (Food and Drug Administration,
Cincinnati, Ohio), *Associate Referee*

In accordance with the recommendations of Committee D (*This Journal*, **30**: 57) a study was made of the determination of added inorganic materials in phosphated flour.

A modification of the carbon tetrachloride sedimentation method of Gustafson (*This Journal*, **19**: 82, 1936) has given satisfactory recovery of monocalcium phosphate added to one type of flour. By use of separatory funnels and large volumes of carbon tetrachloride, the determination may be made in less than one day of elapsed time. It is essential that the sediment be drawn from the separators with a minimum of solution. This can be accomplished by turning the stop-cock quickly from side to side. The carbon tetrachloride may be reused several times, after filtration, before it is necessary to distill. In this case the weighed filter paper and sediment should be washed with fresh carbon tetrachloride.

METHOD FOR PHOSPHATES IN PHOSPHATED FLOUR

Transfer 20 g of flour to a dry 250 ml separatory funnel, add 200 ml carbon tetrachloride, shake well, let stand 15 min. Draw off sediment with a minimum of soln (2–5 ml) into a dry 125 ml separatory funnel. Again shake 250 ml separatory funnel and draw off as before, after standing 1 hour. Nearly fill the 125 ml separatory funnel, containing sediment, with carbon tetrachloride, shake well, stand 2 hours, draw off sediment with a minimum of soln into a weighed, air-dry, filter paper. Dry at room temperature to constant weight (about 2 hours) and weigh.

COLLABORATIVE STUDY

In order to test the reliability of this method in the hands of other chemists, it was submitted to limited collaborative study. For this purpose straight grade soft winter wheat flour was phosphated, Sample No. 1 containing 0.18 per cent monocalcium phosphate and Sample No. 2, 0.66 per cent.

Table 1.—*Collaborative results*

ANALYST	BLANK (UNPHOSPHATED)	SAMPLE NO 1	SAMPLE NO. 2
		(.18%)	(.66%)
F. H. Collins	0.01	0.18	0.66
	.01	.18	.67
O. S. Keener		.22	.66
		.23	.70
F. J. McNall		.20	.65
		.18	.66

RECOMMENDATION*

It is recommended that study be continued on various types and grades of flour with definite amounts of added monocalcium phosphate.

REPORT ON BROMATES IN WHEAT FLOUR[1]

By W. F. GEDDES (Division of Agricultural Biochemistry, University of Minnesota Agricultural Experiment Station, St. Paul, Minn.),
Associate Referee

The establishment, in 1941, of definitions and standards of identity for bromated flour, enriched bromated flour, and bromated whole wheat flour,[2] effective January 1, 1942, under the provisions of the Federal Food, Drug, and Cosmetic Act of 1938, permitting the presence of not more than 75 p.p.m. of potassium bromate in these bromated products, created a need for an accurate and convenient method for the determination of this ingredient in wheat flour. At its annual meeting in 1943, the Association of Official Agricultural Chemists took cognizance of this need by appointing an Associate Referee on the determination of bromates in flour.[3]

The ideal method for use in the administration of the standards for bromated flour should meet the following requirements:

(1) It should be simple and capable of giving accurate, reproducible results for small amounts of potassium bromate (5 to 75 p.p.m.) in the presence of other oxidizing agents that might possibly be illegally added as flour improvers.

(2) It must determine bromates to the exclusion of bromine, chlorine, and iodine naturally present in flour.

(3) It must be applicable in the presence of bromine-containing residues which result from fumigation with methyl bromide.

To these could be added a further specification that the method should be capable of determining bromate whether it is added to the milled product as a finely divided solid, or as a solution in which the wheat is soaked prior to milling or which is sprayed on clean middlings. However, on the basis of available information, treatment with solutions of potassium bromate has only been applied to a limited extent and is confined to the production of bromated whole wheat flour. As this product represents a relatively small percentage of the total bromated flour produced, it did not appear advisable, at the present stage of the work, to consider the special problems involved in determining bromate added in this manner.

From a survey of the literature it was apparent that the available methods held little promise of meeting all the requirements which have been enumerated. Methods for the micro-determination of bromine in biological materials are not applicable, as they would determine native bromine and bromine containing residues from methyl bromide fumigation. However, several procedures for the determination of bromates in flour have been described and, as a first approach to the problem, Brown[4] made a comparative study of them by analyzing flours containing known quantities of potassium bromate. The most satisfactory method for eliminating the interfering influence of other sources of bromine in the flour involves removal of the added bromate by carbon tetrachloride flotation. The most satisfactory results were obtained by a modification of the Kuhlman[5] method in which this is accomplished through the use of a brass centrifuge tube with a removable base; after separation, the bromate is determined by iodometric titration. This procedure, designated in the present paper as "the direct method," is not applicable in the presence of other oxidizing improvers and a "complete method," involving a combination of standard procedures, was developed which is specific for bromate in the presence of persulfates and iodates (also chlorates, which do not act as flour improvers). In the complete method, the solution of the improvers (separated from the flour by flotation) is boiled to destroy persulfates and then treated with sulfurous acid to reduce bromates and iodates to halides. After elimination of the iodide by boiling with sodium nitrite and sulfuric acid, the bromide is determined by the Kolthoff and Yutzy[6] method, which is applicable in the presence of chlorides.

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* For report of Subcommittee D and action by the Association, see *This Journal*, 31, 59 (1948).

[1] Paper No. 2359, Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2] *Federal Register*, 6 (63), 1734 (1941).

[3] *This Journal*, 27, 16 (1944).

The direct method gave mean recoveries ranging between 94 per cent and 102 per cent when applied to straight grade, clear, and whole-wheat flours containing from 5 to 80 p.p.m. of potassium bromate. Similar recoveries were obtained by the complete method with straight grade flours containing these levels of potassium bromate, either added alone or in conjunction with potassium persulfate, potassium iodate, and potassium chlorate. As bromated straight grade flours gave quite satisfactory results by both methods, the two procedures were subjected to a limited series of collaborative tests in 1946. The experience gained from these studies led to slight modifications and the revised methods are given below.

COMPLETE METHOD FOR THE DETERMINATION OF BROMATES IN WHEAT FLOUR

(Applicable in the presence of potassium persulfate and potassium iodate)

APPARATUS

Centrifuge, International type C, equipped with head to carry holders for centrifuge tubes, 1.30"×4.2".

Centrifuge tubes, 2 special brass centrifuge tubes with screw-cap and removable base.

Burettes: one 5 ml (micro), one 50 ml.

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[4] Brown, R. E. The Determination of Bromates in Wheat Flours Containing Various Oxidizing Improvers, M.S. Thesis, University of Minnesota, June 1946.

[5] Kuhlman, J., *Z. Untersuch. Lebensm.* 68, 375 (1934). Original not seen; C.A. 29, 1890 (1935); Chem Listy, 28, 241 (1934). Original not seen; C.A. 29, 236 (1935).

[6] Kolthoff, I. M., and Yutzy, H., *Ind. Eng. Chem., Anal. Ed.*, 9, 75 (1937).

Erlenmeyer flasks: 250 ml.
Funnels: glass, 7.5 cm.

REAGENTS

(1) *Ammonium molybdate soln*, 2.5%.
(2) *Carbon tetrachloride*, technical.
(3) *Potassium iodide*, C.P.
(4) *Sodium bicarbonate*, C.P.
(5) *Sodium formate soln*, 50%.
(6) *Sodium dihydrogen phosphate, monohydrate* C.P.
(7) *Sodium hypochlorite soln*, 1 *N* in approx. 0.1 *N* NaOH. Pass 71 g of chlorine into a cold soln containing 88 g of sodium hydroxide in 1500 ml distilled water (keep the alkali soln cool by means of an ice-salt mixture). After addition of the chlorine, dilute the soln to 2 liters. The alkalinity should be tested at intervals by destroying the hypochlorite in 1 ml of the soln with 2 ml of 3% hydrogen peroxide soln, diluting to 10 ml and titrating with 0.1 *N* hydrochloric acid soln. The titration volume should be between 0.8 and 1.2 ml. This soln is stable for several weeks if stored in a dark bottle at ice-box temperature. (If desired, a smaller quantity of this solution may be prepared.)
(8) *Sodium nitrite soln*, 0.5 *M*.
(9) *Sodium thiosulfate soln*, 0.005 *N*. Prepare from a stock 0.1 *N* soln and standardize just before use, preferably against potassium bromate.
(10) *Starch indicator, 0.5% soln.* Triturate one g of soluble starch and 5 mg of mercuric iodide (preservative) in a small quantity of cold water and pour slowly into about 150 ml of boiling water with constant stirring. Boil until the soln is clear. Cool, and dilute to approximately 200 ml.
(11) *Sulfuric acid solution*, 1 *N*.
(12) *Sulfurous acid solution*, 5%.
(13) *Sulfuric acid*, 12 *N*.

PROCEDURE

The base of the brass centrifuge tube is tightened by placing in position on the base plate, gripping the body with a pliers and applying moderate force. Pour about 10 ml of carbon tetrachloride into the tube followed by 20 g of flour, then nearly fill with carbon tetrachloride. To guard against trapping an excessive amount of air in the tube it is advisable to agitate the flour mass with a small glass rod while adding the second portion of carbon tetrachloride, taking care to rinse down the glass rod. Screw on the cap of tube and shake vigorously for one minute to insure suspension of the flour and wetting of all bromate particles. Centrifuge at 2400 r.p.m. for two minutes. No centrifuge tube holder is necessary, since the flange provides for suspension of the tube without the use of an auxiliary holder. Hold the tube over a suitable receptacle for the excess carbon tetrachloride and screw off the base. As the threads release the base it is moved to the side and the excess carbon tetrachloride flows from the body of the tube. The particles of bromate are packed firmly in the base of the tube and are not disturbed by motion of the liquid created by removing the base. The flour remains in the tube in the form of a plug. Remove most of the carbon tetrachloride from the cup-like base by means of a cotton swab and allow the remainder to evaporate at room temperature (see Note 1). Transfer the residue to a 250 ml Erlenmeyer flask by holding the cup over a 7.5 cm funnel, and wash out the bromate with about 30 ml of water. Wash and dry the base and attach it to the body of the tube. Resuspend the flour in carbon tetrachloride and repeat the flotation separation, removing the excess carbon tetrachloride and transferring the bromate to the same Erlenmeyer flask in the manner described above.

Boil the soln of oxidizing improvers for 3 min. to destroy any persulfates that may be present. Add 5 ml of 1 *N* sulfuric acid soln and 2 ml of 5% sulfurous acid soln. Allow to stand 1 min. and boil briefly to expel excess sulfur dioxide. Add 0.25 ml of 0.5 *M* sodium nitrite soln and boil for 2 min. after the disappearance of the iodine color (see Note 2). Cool the soln (preferably in an ice bath) and add one g of sodium bicarbonate (see Notes 3 and 4). Add one g of sodium dihydrogen phosphate followed by 5 ml of the sodium hypochlorite soln (1.0 *N* in 0.1 *N* NaOH), and heat just to boiling. After about one min., add 5 ml of the 50% sodium formate soln and boil for 2 min. After cooling to room temperature, dilute to about 150 ml and add 0.5 g of potassium iodide, one drop of 2.5% ammonium molybdate soln, 10 ml of 12 *N* sulfuric acid, and titrate immediately with 0.005 *N* sodium thiosulfate soln.

Carry out a blank determination on the reagents, substituting 10 ml of distilled water for the test soln and carry out the procedure as outlined above starting with the addition of sulfuric and sulfurous acid.

Calculate the results in terms of p.p.m. potassium bromate in the sample as follows:

Potassium bromate (p.p.m.) = Normality of $Na_2S_2O_3$ × (titration volume-blank ×) 1392.

DIRECT METHOD FOR THE DETERMINATION OF BROMATES IN WHEAT FLOUR

(Not applicable in the presence of other oxidizing flour improvers)

PROCEDURE

Separate the bromate and other improvers which may be present by the double flotation procedure described in the complete method. To the aqueous bromate soln, add 0.5 g potassium iodide, one drop of 2.5% ammonium molybdate soln, 10 ml of 12 *N* sulfuric acid soln, and titrate with 0.005 *N* thiosulfate.

Calculate the results as follows:

Potassium bromate (ppm) = Normality ($Na_2S_2O_3$) × titration volume × 1392.

Notes

(1) Evaporation of residual carbon tetrachloride is hastened by rotating the cuplike base between thumb and forefinger and breathing gently on the liquid.

(2) When boiling the soln after the addition of the 0.5 *M* sodium nitrite soln, care must be taken to shake the flask continuously. A 3-min. boiling time is sufficient if the volume of the soln is kept small (ca 20 ml), but a longer time is required if the volume is appreciably increased. With samples containing only $KBrO_3$, no iodine coloration will be observed. Boiling here serves to destroy excess nitrous acid.

(3) It is important to cool the soln after the nitrite treatment before proceeding with the hypochlorite oxidation to avoid low results. It seems plausible that the sodium hypochlorite, being somewhat unstable, may be decomposed before the oxidation of the bromide is complete.

(4) The use of indicators in the neutralization makes for low recoveries.

(5) With samples containing from 10 to 75 p.p.m. potassium bromate, the titration volumes will vary from approximately 1 to 12 ml and burettes should be selected accordingly. With samples containing small quantities of added bromate, flotation separations should be made on two subsamples and the material combined in order to secure a larger titer. (In this case, the factor 696 is used in calculating the results.)

COLLABORATIVE STUDY OF REVISED METHODS

Preparation of Samples and Instructions to Collaborators

In 1947, nine chemists employed the revised methods in analyzing four samples, lettered A to D inclusive (Table 1). Samples A, B, and C

TABLE 1.—*Results obtained by each collaborator in the determination of potassium bromate in straight grade flour*

ANALYST NO.	DIRECT METHOD				COMPLETE METHOD			
	MIN.	MAX.	MEAN	MEAN RECOVERY	MIN.	MAX.	MEAN	MEAN RECOVERY
	p.p.m.	*p.p.m.*	*p.p.m.*	*Per cent*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*Per cent*
SAMPLE A (containing 5.0 p.p.m. of potassium bromate)								
1	4.8	5.2	5.0	100	4.4	5.2	4.8	96
2	4.5	5.7	5.1	103	3.7	4.2	4.0	81
3	4.4	5.3	5.0	100	2.4	3.2	3.0	61
4	4.5	5.2	4.8	96	3.9	4.8	4.3	86
5	3.9	4.0	3.9	78	3.2	5.3	4.4	88
6	4.3	5.2	4.8	96	0.8	2.0	1.5	30
7	4.1	5.2	4.6	91	4.1	10.8	6.5	130
8	3.5	4.2	3.8	76	4.0	4.5	4.2	84
9	4.9	5.7	5.2[1]	104	2.2	4.3	3.7	74
All	3.5	5.7	4.7	94	0.8	10.8	4.0	81
SAMPLE B (containing 40 p.p.m. of potassium bromate)								
1	34.9	39.2	37.7	94	36.0	40.0	37.9	95
2	36.7	40.1	38.5	96	34.0	39.6	37.6	94
3	35.8	37.9	37.0	92	29.4	32.7	30.8	77
4	39.3	43.3	40.6	101	35.4	41.3	37.7	94
5	36.7	39.0	38.2	96	20.7	32.4	28.2	71
6	36.2	41.5	39.2	98	16.3	23.4	20.1	50
7	37.2	42.5	39.2	98	17.4	23.0	20.2	50
8	40.4	44.5	43.0	108	32.7	36.2	34.3	86
9			40.3[2]	101	27.6	39.6	35.6	89
All	35.8	44.5	39.2	98	16.3	41.3	31.4	78
SAMPLE C (containing 75 p.p.m. of potassium bromate)								
1	71.7	72.4	72.0	96	68.2	74.6	70.8	94
2	74.2	76.9	75.6	101	69.0	74.2	70.7	94
3	70.3	76.1	72.4	96	64.7	67.9	66.4	88
4	68.5	77.1	73.6	98	64.0	74.2	69.7	93
5	69.0	73.1	71.5	95	49.4	67.1	58.8	78
6	73.1	78.1	76.5	102	42.5	54.6	49.7	66
7	66.1	81.8	72.4	96	27.8	51.5	34.8	46
8	76.5	79.3	78.1	104	68.2	70.3	68.9	92
9	73.1	78.0	75.2	100	76.2	78.9	77.6	104
All	66.1	81.8	74.1	99	27.8	78.9	64.0	85
SAMPLE D (containing 40 p.p.m. potassium bromate, 40 p.p.m. potassium persulfate, and 20 p.p.m. potassium iodate)								
1					36.6	41.8	39.5	99
2					35.8	40.5	37.8	95
3					49.1	52.4	50.7	127
4					60.7	67.8	64.1	160
5					30.6	36.9	34.6	86
6					20.9	27.4	24.6	61
7					21.6	26.1	23.8	60
8					64.0	65.4	64.5	161
9					39.3	44.5	42.2[2]	106
All					20.9	26.1	43.7	109

[1] Mean based on 3 determinations.

[2] Mean based on 2 determinations.

contained 5, 40, and 75 p.p.m. respectively, of potassium bromate, while sample D contained 40 p.p.m. potassium bromate, 40 p.p.m. potassium persulfate, and 20 p.p.m. potassium iodate. In preparing the samples, analytical reagent grade chemicals ground to pass a standard U. S. 100-mesh sieve, and an unenriched, straight grade, hard red spring wheat flour (13.0% protein, 0.47% ash, at 12.8% moisture), which had been bleached with benzoyl peroxide, were employed. A "Master Mix" containing 1000 p.p.m. of potassium bromate was first prepared by making successive additions of flour and bromate to a small MacClellan batch mixer to give a total weight of 3600 g, and mixing for one hour at 5 r.p.m. This master mix was then diluted with the appropriate quantities of flour to give the desired bromate levels in samples A, B, and C, each sample being again mixed for one hour. In preparing sample D, the other oxidizing improvers were added to the flour used for diluting the master mix. Approximately 300 grams of each sample were supplied the analysts to provide sufficient material for preliminary determinations.

The collaborators were requested to analyze samples A, B, and C in quadruplicate by both the direct and complete procedures, and sample D by the complete procedure. In order that a valid measure of the experimental error might be obtained, it was requested that, after experience had been gained with the methods, quadruplicate tests should be made and reported without elimination of any of the results, unless it was definitely known that accidental errors had occurred. Two pairs of the special brass centrifuge tubes were constructed and circulated among the analysts.

Results and Discussion:

The results of the individual analysts for each sample and the means for all collaborators are summarized in Table 1. To ascertain the experimental errors, the replicate values for each sample, excepting those for analyst No. 9, who regarded certain of his replicates as in serious error and suggested that they be eliminated in computing his mean values, were subjected to an analysis of variance. The variance due to the differences between collaborators was significant for each sample by both methods and only the means and standard errors are shown in Table 2.

For the direct method, the recovery of the added bromate by the individual analysts varied from 76 to 103 per cent for sample A, from 92 to 108 per cent for sample B, and from 95 to 104 per cent for sample C; the mean recoveries for all analysts combined were 94, 98, and 99 per cent for the three respective samples. If the low recoveries of 76 and 78 per cent of analysts 5 and 6, for sample A, are eliminated the mean recovery for this sample is increased to 99 per cent. These average results are eminently satisfactory for a microdetermination of this nature. The average reproducibility of the results within laboratories is measured by the standard errors for a single determination shown in Table 2. When these errors are expressed as percentages of the corresponding mean quantities of bromate

TABLE 2.—*Means and standard errors for analysts 1 to 8 inclusive. Direct vs. complete methods for determination of potassium bromate in wheat flour*

SAMPLE	BROMATE ADDED	DIRECT METHOD				COMPLETE METHOD			
		BROMATE FOUND	RE-COVERY	STAND-ARD	ERROR[1]	FOUND	RE-COVERY	STAND-ARD	ERROR[1]
	p.p.m.	*p.p.m.*	*per cent*	*p.p.m.*	*per cent*	*p.p.m.*	*per cent*	*p.p.m.*	*per cent*
A	5.0	4.6	93	0.39	8.5	4.1	82	1.14	27.9
B	40.0	39.2	98	1.83	4.7	30.9	77	2.81	9.1
C	75.0	74.0	99	3.24	4.4	61.2	82	5.82	9.5
D	40.0	—	—	—	—	42.4	106	2.32	5.5

[1] Standard errors given are those computed for a single determination. The percentage values are the standard errors of a single determination expressed as percentages of the corresponding means.

found, they may be employed for a direct comparison of the reproducibility of these determinations with those of others for which similar studies have been made. In a recent statistical study by Anderson[7] of the results of collaborative determinations of several constituents of wheat flour on samples sent out by the National Check Sample Committee of the American Association of Cereal Chemists, it was found that the interlaboratory errors of moisture, protein, and ash determinations were in the order of ±1 per cent, whereas the errors for constituents present in microquantities, such as thiamine, niacin, and iron, ranged between 9 and 13 per cent. The interlaboratory errors of 4.4 to 8.5 per cent for potassium bromate determined by the direct method compare very favorably with the latter group, especially since the analysts participating in the vitamin and iron determinations had previous experience with the methods.

The results by the complete method, however, are quite unsatisfactory. The mean recoveries for all analysts were 81, 78, 85, and 109 per cent for samples A to D, respectively, and the recoveries obtained by individual analysts varied from a low of 30 per cent for Sample A to a high of 161 per cent for Sample D. The interlaboratory errors also greatly exceeded those for the direct method. Nevertheless, several analysts secured quite satisfactory recoveries, and further study is warranted in an effort to trace the sources of the error.

The complete method is not required if only bromates and persulfates, or bromates and iodates are present. In the former case, it is only necessary to boil the solution of the improvers which are removed from the sample by the flotation procedure and titrate in the manner employed in the direct method. In the latter case, bromates and iodates may be titrated in the same solution by the iodometric titration described by Kolthoff and Hume.[8] In this procedure the iodate is first titrated in the presence of a phthalate buffer between *p*H 4 and 5, after which the bromate is titrated upon the addition of an adequate amount of hydrochloric acid and a few drops of a molybdate solution as a catalyst. Alternatively, the bromates and iodates could be reduced to iodide and bromide with sodium nitrite and sulfuric acid and the iodide eliminated as iodine by boiling with sodium nitrite, as described in the complete method. By suitable qualitative tests for the presence of oxidizing improvers other than potassium bromate, simpler analytical procedures could be applied which might give more accurate and reproducible results than the complete method.

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[7] Anderson, J. A., *Transactions Am. Assoc. Cereal Chem.*, 5, 178 (1947).
[8] Kolthoff, I. M., and Hume, D. N., *Ind. Eng. Chem. Anal. Ed.*, 15, 174 (1943).

LIST OF COLLABORATORS[a]

1. A. W. Alcock, Purity Flour Mills, Limited, Winnipeg, Manitoba.
2. J. A. Anderson, Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, Manitoba.
3. Ray Brown, International Milling Co., Minneapolis, Minn.
4. Rowland Clark, W. E. Long Co., Chicago, Ill.
5. John E. Despaul, General Chemistry Branch, Quartermaster Food and Container Institute for the Armed Forces, Chicago, Ill.
6. F. C. Hildebrand, General Mills Inc., Minneapolis, Minn.
7. W. L. Rainey, Commander Larabee Milling Company, Minneapolis, Minn.
8. M. O. Schultze, Division of Agricultural Biochemistry, University of Minnesota, St. Paul, Minn.
9. V. A. Stenger, The Dow Chemical Company, Midland, Michigan.

COMMENTS OF COLLABORATORS

(*1*) The flotation procedure is the weakest point of the methods. There is no absolute assurance that mechanical loss of bromate during the opening of the centrifuge cups can be entirely avoided. Drilling a well in the bottom of the conical portion of the cup might assist in avoiding this difficulty.

(*2*) The special brass centrifuge tubes are very unsatisfactory for separating bromate or other flour improvers from the sample. It was very difficult to obtain a clean separation with these tubes, especially with tube No. 1, which invariably left deposits of flour in the lower portion of the tube. When flour is present in the sample being titrated, the end point is, of course, obscured. We have had much better success with regular Pyrex glass conical centrifuge tubes, which are less expensive, and easier and faster to use.

(*3*) In two trials on Sample D by the complete method values of 51.6 and 52.2 p.p.m. of potassium bromate were obtained with only one flotation, as compared with a mean value of 50.7 for quadruplicate determinations based on the double flotation procedure described. As a better recovery was obtained with a single flotation separation, a further study of the method to eliminate the second flotation is indicated.

(*4*) In some cases, difficulty was encountered in obtaining a compact mass of flour and it is suggested that an extra minute of centrifuging would aid in obtaining a clearer extract.

(*5*) The direct method was fairly easily carried out and the end points in the titrations were fairly good. There may be danger of losing bromate when removing the excess of carbon tetrachloride from the base of the tube by means of a cotton swab, and it is suggested that additional remarks on this manipulation be included in the description of the method.

(*6*) The use of 12 *N* sulphuric acid is not quite strong enough to secure reproducible results. Where more acid was used, better checks were obtained.

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[a] The numbers assigned to the collaborators in Table 1 bear no relation to the alphabetical arrangement in this list.

(*7*) It is believed that sufficient fat is extracted from the flour by the carbon tetrachloride to cause difficulty in the quantitative transfer of the potassium bromate from the cup-like base due to gumminess of the mass after evaporation of the carbon tetrachloride.

(*8*) A sticky deposit in the base of the centrifuge tubes was present after evaporating the carbon tetrachloride, and hot water was used to wash out the tubes to avoid loss of any potassium bromate which might remain undissolved.

(*9*) The procedure should include cooling the boiled solution prior to the addition of sulfurous acid.

(*10*) With the experience gained in completing this group of determinations, it is believed that better checks would be obtained if the work were repeated.

(*11*) The results are not as precise as would be desirable but the methods are probably suitable for indicating whether or not the bromate additions are within the desired range.

(*12*) In the notes, it is stated that the use of indicators in the neutralization makes for low recoveries. Would there be any advantage in carrying through a separate sample containing indicators for the purpose of checking and adjusting the alkalinity or acidity of the sample? If so, what should the reaction be at the various stages of the procedure?[10] The end points in the complete procedure were very poor.

RECOMMENDATIONS*

It is recommended—

(1) That an improved design of the special brass centrifuge tube with separable base be compared with conical Pyrex centrifuge tubes for the determination of potassium bromate in flours by modifications of the direct method.

(2) That no further collaborative studies by the complete method be undertaken until the possibility of developing simpler procedures for the determination of bromate in the presence of one or more additional improvers is investigated.

(3) That qualitative tests on the detection of various oxidizing improvers added to flours, both singly and in combination, be studied.

(4) That methods be outlined for the determination of bromate in solutions containing this improver in the presence of potassium persulfate and potassium iodate, respectively, and that these methods be tested collaboratively with solutions containing known quantities of these improvers.

ACKNOWLEDGMENT

The thanks of the Associate Referee are extended to Ray Brown, International Milling Company, for preparing the samples, assembling and studying the data submitted by the collaborators. His thanks are also extended to the other collaborators for their cooperation.

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[10] It would be of no apparent advantage to carry a separate sample through the procedure for the purpose of adjusting the reaction, since sodium bicarbonate is added in excess. The amount of the excess is relatively unimportant, since the solution is buffered with a large excess of sodium dihydrogen phosphate.

* For report of Subcommittee D and action by the Association, see *This Journal*, **31**, 59 (1948).

REPORT ON PHOSPHORUS IN CEREAL PRODUCTS

By V. E. MUNSEY (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

The Official and Tentative Methods of Analysis, Sixth Edition, does not contain a method for the determination of phosphorus in either the Cereal Foods or the Grain and Stock Feed chapters. Two adaptations of official methods from the Plant chapter have been applied to various cereal products with satisfaction by the Associate Referee. Twelve collaborators were invited to analyze four samples, namely, bread, oatmeal, Pablum (an infant cereal food), and self-rising flour, by two new procedures. Samples in Procedure No. 1 are ashed after mixing with magnesium nitrate solution, while Procedure No. 2 requires addition of sodium carbonate, followed by ashing as in the official method for ash in flour. The ash is dissolved by acid and the determination completed in essentially the same manner in both procedures by proceeding as for the determination of phosphorus under Fertilizers, sec. **2.12**, page 23. The details of the methods are given in *This Journal,* **31,** 79, (1948), under "Changes in Methods of Analysis."

The results reported by 12 collaborators are given in Table 1, as follows:

TABLE 1.—*Phosphorus in oatmeal, bread, pablum, and self-rising flour*

COLLABORATOR		BREAD	OATMEAL	PABLUM	SELF-RISING FLOUR
		per cent	*per cent*	*per cent*	*per cent*
1	Procedure I	0.21	0.48	0.65	0.53
		0.20	0.48	0.66	0.54
	Procedure II	0.20	0.48	0.65	0.53
		0.20	0.48	0.65	0.53
2	Procedure I	0.18	0.44	0.67	0.51
		0.19	0.46	0.66	0.50
	Procedure II	0.14	0.45	0.68	0.50
		0.14	0.44	0.66	0.50
3	Procedure I	0.21	0.54	0.78	0.61
	Procedure II	0.19	0.57	0.75	0.58
4	Procedure I	0.15	0.46	0.68	0.53
		0.15	0.46	0.67	—
	Procedure II	0.14	0.46	0.67	0.52
		0.15	0.45	0.68	0.53
5	Procedure I	0.15	0.51	0.67	0.53
	Procedure II	0.15	0.45	0.66	0.52
6	Procedure I	0.16	0.48	0.67	0.54
	Procedure II	0.16	0.48	0.67	0.53

TABLE 1.—(*continued*)

COLLABORATOR		BREAD	OATMEAL	PABLUM	SELF-RISING FLOUR
		per cent	*per cent*	*per cent*	*per cent*
7	Procedure I	0.16	0.47	0.69	0.53
		0.16	0.47	0.68	0.54
	Procedure II	0.16	0.48	0.68	0.53
		0.16	0.48	0.69	0.53
8	Procedure I	0.19	0.50	0.69	0.50
		0.12	0.42	0.66	0.45
		0.15	0.47	0.66	0.51
	Procedure II	0.14	0.45	0.66	0.46
		0.19	0.45	0.65	0.45
		0.16	0.44	0.67	0.45
9	Procedure I	0.160	0.446	0.659	0.533
		0.156	0.442	0.663	—
		0.157	0.478	0.689	0.515
		0.161	0.475	0.695	0.520
		0.163	0.480	0.681	0.521
		0.161	0.479	0.686	0.520
	Procedure II	0.147	0.464	0.676	0.520
		0.156	0.458	0.676	0.524
		0.147	0.468	0.676	0.520
		0.152	0.459	0.672	0.520
		0.151	0.471	0.689	0.523
		0.152	0.472	0.692	0.521
10	Procedure I	0.13	0.44	0.63	0.51
		0.13	0.44	0.64	0.49
	Procedure II	0.13	0.44	0.65	0.49
		0.13	0.44	0.65	0.49
11	Procedure I	0.16	0.47	0.69	0.49
		0.16	0.47	0.69	0.51
	Procedure II	0.16	0.46	0.69	0.50
		0.17	0.47	0.69	0.50
12*	Procedure I	0.14	0.45	0.65	0.51
		0.14	0.45	0.66	0.51
	Procedure II	0.14	0.45	0.66	0.51
		0.14	0.45	0.66	0.51
		Procedure I			
	Min.	0.13	0.44	0.64	0.49
	Max.	0.21	0.54	0.78	0.61
	Av.	0.17	0.47	0.68	0.52
		Procedure II			
	Min.	0.13	0.44	0.65	0.45
	Max.	0.20	0.57	0.75	0.58
	Av.	0.16	0.47	0.67	0.51

* Results received after averages were made.

Collaborator 2 uses for routine analysis of flour, phosphated and self-rising flour, a method essentially that published by H. R. Allen and Lelah Gault, *This Journal*, **30,** 136 (1947), Method C. This method specifies nitric-perchloric acid digestion, requires a relatively short time of about 2½ hours and offers smaller chance for error than the two procedures submitted for collaboration. His results (% P) obtained by this method follow:

Bread	*Oatmeal*	*Pablum*	*Self-rising Flour*
0.14	0.46	0.67	0.52
0.14	0.46	0.67	0.52

Collaborator 9 reports on the use of a colorimetric method, with slight modification, of the one described by Kitson and Mellon in *Ind. Eng. Chem., Anal. Ed.*, **16,** 379, 1944. Their method is less time-consuming than the procedures submitted, and while the results obtained were slightly lower, the differences were not considered significant. His results (% P) by this procedure follow:

Bread	*Oatmeal*	*Pablum*	*Self-rising flour*
0.148	0.460	0.668	0.520
0.144	0.451	0.652	0.495
0.140	0.450	0.655	0.503
0.136	0.455	0.663	0.510
0.143	0.450	0.660	0.505

Collaborator 8 submitted results obtained by a method regularly used by them, although details of the method were not given. The results (% P) are:

Bread	*Oatmeal*	*Pablum*	*Self-rising flour*
0.14	0.45	0.65	0.50
0.14	0.46	0.64	0.51

Collaborator 11 obtained the following results by Procedure II with the exception that the samples were ashed as in the official method for ash in flour, that is, without a fixative (% P):

Bread	*Oatmeal*	*Pablum*	*Self-rising flour*
0.16	0.47	0.69	0.55
0.16	0.47	0.69	0.55

Collaborator 6 points out the possibility of getting high results, especially with small amounts of P_2O_5, an account of deterioration of the molybdate solution. He recommends the molybdate solution, *Methods of Analysis*, **26.46 (a)** p. 398, which is stable and does not give high results. Results (% P) obtained with this solution follow:

Bread	*Oatmeal*	*Pablum*	*Self-rising flour*
0.16	0.48	0.67	0.53
0.16	0.48	0.68	0.53

The variations and the averages by the two procedures submitted for collaborative study are essentially the same on all four samples. No difficulties were experienced. These procedures are an adaptation of methods already official for other products. The collaborative results indicate both procedures are satisfactory for cereal products. The results obtained by the other procedures referred to, in regular use by some collaborators, are also in close agreement. The advantages of a nitric-perchloric acid digestion are not of sufficient magnitude to overcome the difficulty and danger of its application to warrant a collaborative study.

The assistance of the collaborators is gratefully acknowledged:

J. J. Winston, Jacobs Cereal Products Laboratory, New York City
E. R. Winterlee, Victor Chemical Works, Chicago Heights, Ill.
F. H. Luckmann, The Best Foods, Inc., Bayonne, N. J.
L. C. Andrews, Department of Health, New Orleans, La.
T. W. Schilb, Monsanto Chemical Co., St. Louis, Mo.
G. W. Kirby, The Fleischmann Laboratory, New York City
H. C. Randall, Rumford Chemical Works, Rumford, R. I.
J. F. Armstrong, M. J. Gnagy, Daniel Banes, and H. W. Conroy—all of the Food & Drug Administration.

It is recommended* that both procedures be adopted as official, first action, for cereals and cereal products and that the study be continued.

REPORT ON MILK SOLIDS AND BUTTERFAT IN BREAD

By V. E. MUNSEY (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

Further attempts have been made to apply the method in sec. **20.122**, p. 266, *Methods of Analysis*, to eliminate extraction of the fat. In a modification of this procedure, the saponified bakery product was acidified with sulfuric acid and steam distilled. The distillate was treated for application to the column as discussed in the paper by Ramsey & Patterson (*This Journal*, **28**, 644 (1945). There was found a relatively large percentage of several acids formed from the action of strong potassium hydroxide on the starch and sugar so that the separation of butyric acid on the silicic acid column was complicated to such an extent that the effort required in preparation for the column did not seem to offer a practical procedure.

Another approach was to extract the fat from bread with and without added butterfat in accordance with tentative method, sec **20.86**, p. 261, up to the point where the distillation is carried out as on a Reichert-Meissl determination. Instead of that distillation, the sample was steam distilled and the distillate prepared for separation on a silicic acid column according to the above reference. The breads containing no butterfat contained 0–0.9 mg butyric acid per g of fat, whereas the fat from a milk bread contained 15.2 mg butyric acid per 1 g of fat. This procedure gives a means for the direct measurement of butyric acid and may prove to be more desirable than the present procedure using the Reichert-Meissl technic. Further work is necessary and possibly collaborative results. No work was done this year on the determination of lactose for the calculation of nonfat milk solids in bread.

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* For report of Subcommittee D and action by the Association, see *This Journal*, **31**, 58 (1948).

It is recommended* that study be continued on (1) the determination of lactose in bread and (2) on the determination of milk fat in bread.

No report was given on the following subjects: proteolytic activity of flour; soybean flour; soybean flour (immunological tests); noodles; baked products (moisture, ash, protein, fat, and crude fiber); moisture in self-rising flour and pancake, waffle, and doughnut flours; apparent viscosity measurement; iron in cornmeal and macaroni products.

REPORT ON BAKING POWDER AND BAKING CHEMICALS

By V. E. MUNSEY (Food and Drug Administration, Federal Security Agency, Washington D. C.), *Referee*

The gasometric method, official, for residual CO_2 (sec. **17.8**, p. 210), does not give the results expected, and on one type of baking powder the results are high to such an extent that the method does not seem applicable. The Associate Referee reports collaborative results this year, which confirm this statement and also presents an improved method to replace the official method.

RECOMMENDATIONS†

The Referee concurs in the following recommendation of the Associate Referee on Baking Powder:

It is recommended—

(1) That the present official method for determination of residual carbon dioxide in baking powders be dropped (first action), for the following reasons:

(a) The wording and description of the method are susceptible to too many interpretations.

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* For report of Subcommittee D and action by the Association, see *This Journal*, **31**, 58 (1948).
† For the report of Subcommittee D and action by the Association, see *This Journal*, **31**, 59 (1948).

(b) The manner of heating and treating the sample does not yield a true residual because all of the available CO_2 is not driven off.

(c) The method is not applicable to all types of baking powder, particularly the pyrophosphate types.

(2) That the modified Quartermaster Corps method as described in the report of the Associate Referee, this year, be adopted as a tentative method, adding the words "or electricity" after the phrase "The bath should be heated with gas."

(3) That the modified Q. M. C. method referred to in recommendation (2) be subjected to further study wherein the single evaporation to dryness is carried out in a moisture oven instead of on a water bath and at temperatures ranging from 70 to 100°C.

(4) That the modified Q. M. C. method referred to in recommendation (2) be also studied using a neutral saturated sodium chloride solution as medium instead of distilled water.

(5) That the expression "or subtract 17.8 from 17.6" in method **17.9** Available Carbon dioxide—official, be deleted, and that the tentative available CO_2 be determined by subtraction of the tentative residual CO_2 from the total CO_2, official **17.6.**

(6) That an investigation be made on modifying the present A.O.A.C. gravimetric method, **17.2** and **17.3**, by changing from H_2SO_4 and KOH absorption bulbs to the use of "Caroxite" or "Ascarite and Anhydrone."

REPORT ON RESIDUAL CARBON DIOXIDE IN BAKING POWDERS

By J. E. TATAR (Standard Brands, Inc., Chicago, Ill.), *Associate Referee*

The last report[1] of collaborative work on the determination of residual carbon dioxide in baking powders indicated that the official method, (*Methods of Analysis*, 6th ed., 1945, page 210, sec. **17.8**), needed study with special attention to the time and method of heating in the treatment of the sample.

A collaborative study was instituted involving the services of eleven analysts representing manufacturers of baking powder and leavening chemicals, also research and Federal laboratories. In this work the official method was compared with the method described in the Quartermaster Corps Tentative Spec. C.Q.D. #326, May 24, 1946, and also a modification of the latter method wherein the sample is subjected to a double evaporation instead of the single evaporation outlined.

The three methods were tried on three baking powders which were marked A, B, and C.

A.—S.A.S.—Phosphate Type with starch only as a filler.

B.—S.A.S.—Phosphate Type with the same percentages of S.A.S., phosphate, and sodium bicarbonate as powder A, except that part of the starch was replaced with calcium carbonate.

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[1] *This Journal*, **29**, 259 (1946).

C.—Pyrophosphate-Mono Calcium Phosphate Type baking powder.

FORMULAS OF THE BAKING POWDERS SUBMITTED

Sample Marked "A":

	per cent
Sodium Aluminum Sulphate	24.00
Mono Calcium Phosphate	12.40
Sodium Bicarbonate	31.20
Cornstarch	32.40

Sample Marked "B":

Sodium Aluminum Sulphate	24.00
Mono Calcium Phosphate	12.40
Sodium Bicarbonate	31.20
Cornstarch	16.23
Calcium Carbonate	16.17

Sample Marked "C":

Sodium Acid Pyro Phosphate	20.00
Mono Calcium Phosphate	20.00
Sodium Bicarbonate	28.57
Cornstarch	31.43

METHODS

Official A.O.A.C. Method

Methods of Analysis, 6th Edition (1945), page 210, sec. 17.8; or 5th Edition (1940), page 188.

Quartermaster Corps Method

Quartermaster Corps Tentative Specifications C.Q.D. #326, May 24, 1946.

Residual Carbon Dioxide.—Place 1.7 g of baking powder in a clean, dry, 250 ml wide-mouthed soxhlet extraction flask.* Add 20 ml distilled water. Evaporate to dryness on a steam bath in which the boiling water is kept at a constant level of 2 inches below the top of the bath. The flask must be set into an opening 3 inches in diameter. After 2 hours remove from the steam bath, add 10 ml distilled water and let stand until the flask is at room temperature. Determine carbon dioxide present with Chittick apparatus as described in *Methods of Analysis of the A.O.A.C.*, Fifth Edition, page 186, using correction factors given in Table 24. To obtain the available carbon dioxide subtract the residual carbon dioxide from the total carbon dioxide.

(The determination of the carbon dioxide referred to above in the 5th Edition of the A.O.A.C. *Methods of Analysis*, page 186, is the same as that found in the 6th Edition, pages 208–209.)

Modified Quartermaster Corps Method.—Place 1.7 g of baking powder in a clean, dry, 250 ml wide mouthed soxhlet extraction flask.* Add 20 ml distilled water. Evaporate to dryness on a steam bath in which the boiling water is kept at a con-

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* The 250 ml. wide-mouthed soxhlet extraction flask specified in the two Q.M.C. methods is the same as the decomposition flask used in the A.O.A.C. Official Gasometric Method.

TABLE 1.—*Results of collaborators on sample A in per cent carbon dioxide*

COLLABORATOR	OFFICIAL A.O.A.C. METHOD	DEVIATION FROM THE MEAN	Q.M.C. METHOD	DEVIATION FROM THE MEAN	MODIFIED Q.M.C. METHOD	DEVIATION FROM THE MEAN
1	1.23	.30	.88	.17	.32	.15
	1.05	.12	.81	.10	.36	.11
	1.27	.34	.84	.13	.47	.00
2	.605	.32	.344	.37	.203	.27
	.605	.32	.443	.27	.304	.17
	.705	.22	.443	.27	.304	.17
3	.782	.15	.677	.03	.581	.11
	.703	.23	.657	.05	.678	.20
	.703	.23	.657	.05	.678	.20
4	1.36	.43	.67	.04	.52	.05
	1.30	.37	.72	.01	.52	.05
	1.30	.37	.77	.06	.57	.10
5	.98	.05	1.15	.44	.41	.06
	.98	.05	1.10	.39	.62	.15
	1.04	.11	1.15	.44	.62	.15
6	.89	.04	.55	.16	.41	.06
	.76	.17	.55	.16	.47	.00
	.97	.04	.61	.10	.41	.06
			.56	.15		
7*	2.00	1.07	3.20	2.49	2.80	1.70
	2.10	1.17	2.20	1.49	2.40	1.30
	1.70	0.77	2.60	1.89	2.00	0.90
8	.93	.00	.95	.24	.75	.28
	1.00	.07	.90	.19	.66	.19
	1.05	.12	1.06	.35	.61	.14
9	.87	.06	.57	.14	.41	.06
	.77	.16	.57	.14	.46	.01
	.87	.06	.62	.09	.41	.06
10	.90	.03	.59	.12	.40	.07
	.90	.03	.50	.21	.50	.03
	.70	.23	.61	.10	.45	.02
	.99	.06	.62	.09	.48	.01
	1.07	.14	.67	.04	.34	.13
	.97	.04	.67	.04	.48	.01
	.58	.35				
	.78	.15				
	.77	.16				
11	.97	.04	.81	.10	.40	.07
	1.02	.09	.71	.00	.40	.07
	1.08	.15	.76	.05	.40	.07
Mean	.93		.71		.47	
Ave. Dev.		.16		.15		.10
Max. Var.	.78		.81		.55	

* Results for Collaborator #7 not included in computation of the mean or the average deviation, because the deviations from the mean of these results are greater than 4 times the average deviation.

TABLE 2.—*Results of collaborators on sample B in per cent carbon dioxide*

COLLABORATOR	OFFICIAL A.O.A.C. METHOD	DEVIATION FROM THE MEAN	Q.M.C. METHOD	DEVIATION FROM THE MEAN	MODIFIED Q.M.C. METHOD	DEVIATION FROM THE MEAN
1	7.23	.04	7.18	.32	6.77	.14
	7.30	.03	7.18	.32	6.88	.25
	7.43	.16	7.06	.20	6.87	.24
2	7.00	.27	6.64	.22	6.24	.39
	6.95	.32	6.49	.37	6.34	.29
	7.06	.21	6.59	.27	6.39	.24
3	7.23	.04	7.18	.32	6.88	.25
	7.23	.04	7.06	.20	6.98	.35
	7.13	.14	7.16	.30	6.93	.30
4	7.66	.39	6.79	.07	6.76	.13
	7.77	.50	6.79	.07	6.76	.13
	7.77	.50	6.69	.17	6.76	.13
5	7.73	.45	7.79	.93	6.89	.26
	7.52	.25	7.79	.93	6.99	.36
	7.52	.25	7.73	.87	6.88	.25
6	7.18	.09	7.02	.16	6.86	.23
	7.17	.10	7.07	.21	6.89	.26
	7.13	.14	7.21	.35	6.76	.13
			6.99	.13		
7	7.40	.13	6.90	.04	6.70	.07
	7.70	.43	6.90	.04	6.70	.07
	8.00	.73	7.20	.34	6.70	.07
8	7.44	.17	7.36	.50	7.02	.39
	7.46	.19	7.27	.41	6.93	.30
	7.55	.28	7.31	.45	6.80	.17
9	7.32	.05	6.94	.08	6.62	.01
	7.21	.06	6.84	.02	6.72	.09
	7.21	.06	6.94	.08	6.67	.04
10	6.60	.67	5.84	1.02	6.06	.57
	6.60	.67	5.87	.99	6.16	.47
	6.79	.48	5.88	.98	5.95	.68
	7.08	.19	6.41	.45	5.84	.79
	7.13	.14	5.96	.90	5.60	1.03
	7.01	.21	6.13	.73	5.87	.76
	6.74	.53	6.13	.73		
	6.73	.54				
11	7.49	.22	6.87	.01	6.94	.31
	7.54	.27	6.79	.07	6.92	.29
	7.24	.03	6.80	.06	6.97	.34
Mean	7.27		6.86		6.63	
Ave. Dev.		.23		.37		.27
Max. Var.	1.40		1.95		1.42	

stant level of 2 inches below the top of the bath. The flask must be set into an opening 3 inches in diameter. After 2 hours add 20 ml distilled water and evaporate to dryness in like manner. Add 10 ml distilled water and let stand until the flask is at room temperature. Determine carbon dioxide present with Chittick apparatus as described in *Methods of Analysis of the A.O.A.C.*, 6th Edition, pages 208–209, using correction factor in Table 44.30.

The final carbon dioxide in all three methods was determined in the same way employing the gasometric method described on pages 208–209, secs. 17.4–17.6, inclusive, using 10 ml of hydrochloric acid (1+2) instead of the sulphuric acid (1+5) specified.

Because several of the collaborators raised questions as to interpretation of the directions in the Q. M. C. Method, the Referee and Associate Referee thought it advisable to submit a more detailed version of the method, which follows:

QUARTERMASTER CORPS METHOD FOR DETERMINATION OF RESIDUAL CARBON DIOXIDE IN BAKING POWDERS

(Rewritten Version)

Place 1.7 g of baking powder in a clean, dry, 250 ml wide mouthed soxhlet extraction flask, such as is used in the Offiical A.O.A.C. Gasometric Method. Add 20 ml distilled water. Put the flask *on the cover* of a water bath (single or multiple) in which the boiling water is kept at a constant level of 2 inches below the top of the bath. The water in the bath should be boiling vigorously all through the determination and the bath should be heated with gas. The opening in the cover of the bath into which the flask is set must be 3 inches in diameter. This prevents the flask from touching the water and keeps it a definite distance above it. Evaporate the contents of the flask to *dryness*. (By dryness is meant that there is no visible evidence of moisture in the residue or the inside surface of the flask.) If the set-up is functioning properly, this should take from 1½ to 2 hours. In any case, leave the flask on the water bath for a total of 2 hours. If the sample is not completely dry in 2 hours something is wrong with the set-up. After the sample is completely dry, add 10 ml distilled water and let stand until the flask is at room temperature. This will take about 1 hour.

Determine carbon dioxide present with Chittick apparatus as described in *Methods of Analysis*, 5th Edition, page 186, using correction factors in Table 24, or 6th Edition 1945, page 208–209, and using correction factors in Table 44.30.

The residue in the flask at this point is in such a condition that it is difficult to shake out the carbon dioxide. Shake the flask vigorously until further shaking produces no increment in the reading.

The following collaborators contributed their time and effort in this work:

Barackman, R. A., and Winterle, E. R., Victor Chemical Works, Chicago Heights, Ill.
Bryan, C. S., and Randall, H. C., Rumford Chemical Works, Rumford, R. I.
Despaul, J. E., Quartermaster Food & Container Institute, Chicago, Ill.
Dick, Ludwig, Standard Brands, Inc., Chicago, Ill.
Holch, R. D., Jaques Manufacturing Co., Chicago, Ill.
Honzak, Ruth, General Foods Corporation, Chicago, Ill.
Miller, G. E., Fleischmann Laboratories, New York, N. Y.
Morck, R. A., R. B. Davis Co., Hoboken, N. J.
Munsey, V. E., Food & Drug Administration, Washington, D. C.

TABLE 3.—*Results of collaborators on sample C in per cent carbon dioxide*

COLLABORATOR	OFFICIAL A.O.A.C. METHOD	DEVIATION FROM THE MEAN	Q.M.C. METHOD	DEVIATION FROM THE MEAN	MODIFIED Q.M.C. METHOD	DEVIATION FROM THE MEAN
1	1.22	.28	.36	.02	.00	.17
	1.31	.37	.55	.21	.05	.12
	1.28	.34	.45	.11	.29	.12
2	.655	.28	.295	.04	.101	.07
	.504	.44	.344	.00	.101	.07
	.705	.23	.197	.14	.203	.03
3	.782	.14	.249	.09	.194	.02
	.733	.21	.249	.09	.194	.02
	.782	.16	.348	.01	.290	.12
4	1.04	.10	.31	.03	.16	.01
	1.04	.10	.36	.02	.16	.01
	.99	.05	.31	.03	.16	.01
5	.99	.05	.94*	.60*	.41	.24
	1.05	.11	.94*	.60*	.52	.35
	1.05	.11	.94*	.60*	.41	.24
6	.77	.17	.18	.16	.03	.14
	.85	.09	.25	.09	.10	.07
	.82	.12	.39	.05	.07	.10
			.20	.14		
7	3.30*	2.36*	.50	.16	.30	.13
	3.30*	2.36*	.50	.16	.50	.33
	2.10*	1.16*	1.00*	.66*	.30	.13
8	.58	.36	.25	.09	.15	.02
	.81	.13	.31	.03	.22	.05
	.52	.42	.43	.09	.20	.03
9	1.59	.65	.31	.03	.00	.17
	1.48	.54	.31	.03	.10	.07
	1.53	.59	.26	.08	.05	.12
					.05	.12
					.10	.07
					.05	.12
10	1.20	.26	.54	.20	.17	.00
	.92	.02	.47	.13	.05	.12
	1.40	.46	.23	.11	.02	.15
	.61	.33	.40	.06	.19	.02
	.35	.59	.35	.01	.13	.04
	.81	.13	.34	.00	.13	.04
	.63	.31	.32	.02	.10	.07
	.68	.26	.31	.03	.19	.02
	.96	.02	.48	.14	.19	.02
			.38	.04	.15	.02
			.48	.14	.10	.07
					.20	.03
11	.92	.02	.30	.04	.20	.03
	1.07	.13	.30	.04	.25	.08
	1.17	.23	.35	.01	.20	.03
Mean	.94		.34		.17	
Ave. Dev.		.25		.08		.09
Max. Var.	1.24		.37		.52	

* Results not included in computation of the mean or the average deviation.

Schilb, T. W., and McKim, Elizabeth, Monsanto Chemical Co., St. Louis, Mo.
Stephenson, N. R., Standard Brands Limited, Toronto, Canada.

(The order in which these collaborators are listed does not conform to the numbers used in the tables of results.)

COMMENTS OF COLLABORATORS

Barackman, R. A., and Winterle, E. R.—"We prefer the Quartermaster Corps method for residual CO_2 determinations. The Q. M. C. method, however, does not take into consideration that drying of the sample may be carried too far. The caked residue which is obtained may be thought of as like the crust of a baked product whereas the interior should be simulated if possible. Some tests which we carried out indicated that if a watch-glass is placed on the mouth of the decomposition flask during the two hour heating period on a boiling water bath, the residue will remain as a gel if starch is present. We offer this suggestion for future work."

Schilb, T. A., and McKim, Elizabeth.—"We were unable to get satisfactory checks on the official A.O.A.C. We feel that this is because it is impossible to regulate the time of actual boiling of the sample with a sufficiently high degree of accuracy."

Morck, R. A.—"We have used the Q.M.C. procedure some here in our laboratory and like it very much. While the results are usually lower than those obtained by the A.O.A.C. method, we have found them more consistent. We saw no advantage in the modified procedure over the regular Q.M.C. procedure and of course it does require a great deal more time."

Bryan, C. S., and Randall, H. C.—"We prefer the Quartermaster Corps method to the other two methods for the following reasons:

1. The instructions are more concise than those for the A.O.A.C. method.
2. The A.O.A.C. method, although giving results in the shortest elapsed time, requires more of the attention and time of the operator. For instance, at the point where the method calls for bringing the sample to a quick boil and boiling for one minute, much flame adjusting must be made, for if brought too rapidly to a boil the sample foams up despite the addition of 3 drops of capryl alcohol.
3. The modified Quartermaster Corps method seems to us to be unnecessarily prolonged and to give, in the case of two of the samples, especially sample C, residual CO_2's which seem to us to be very low."

Holch, R. D.—"Evaporation to dryness is a severe condition which is not encountered during actual baking procedure. Evaporation to dryness consumes much more time than the A.O.A.C. method and the reproducibility is not improved."

Stephenson, N. R.—"The official A.O.A.C. method gave higher values than either of the Q.M.C. methods. However, this procedure yields more variable results. A considerable error is introduced into each method unless the Chittick flask is shaken until there is no further increment in the reading of the gas burette."

DISCUSSION

The maximum variances and average deviations of the results in Table 1 on powder A indicate that there is not much difference in precision between the official and Q.M.C. methods. The modified Q.M.C. results have a slight edge in precision, although the precision of all three methods was satisfactory for a determination of this kind. Note that one collaborator had trouble with all three methods on powder A.

The results in Table 2 on powder B are not very good with any of the three methods. This may be due to the calcium carbonate in the formula which brings the results up to such a relatively high level that if the error is partially factorial this would account for the greater maximum variances and average deviations. Calcium carbonate reacts with the acids in the baking powder yielding higher availables by as much as 1.00 per cent in powder B over powder A. Does this mean that powder A is compounded on the acid side and does not really have an excess of soda? At any rate the presence of the calcium carbonate further complicates this determination because it introduces additional reactions.

The results in Table 3 on powder C demonstrate that both Q.M.C. methods are superior to the official method and the precision is probably as good as we ever hope to attain in a determination of this type. Collaborator #7 had trouble with the official method on this powder while #5 had trouble with the Q.M.C. method. On the whole it appears that the precision of the two Q.M.C. methods is at least as good as that of the official method on powders A and B and definitely superior on powder C. It is certainly a disappointment to see that the simple and thoroughly descriptive instructions given in the Q.M.C. methods (particularly the rewritten version) did not produce better agreement in results than the official method on powders A and B. The wording and description of the official method is certainly not up to the standard set by the A.O.A.C. Every analyst has his own interpretation of a "metal drying cell" and the most critical point in the determination "boiling for 1 minute" is very difficult to control and standardize.

Note that there is a difference in the level of the results on all three powders by all three methods, the greatest difference being on powder C where the mean by the official method is 0.94 per cent and 0.34 per cent by the Q.M.C. method. This difference in level is of course due to the difference in time and method of heating. Two of the collaborators comment that the evaporation to dryness in the Q.M.C. method is too severe and is a condition which does not simulate actual baking. This objective of trying to simulate baking conditions with a method of this kind has some merit, but we must remember that distilled water as a medium for the reaction certainly does not even approximate dough conditions where you have a large mass of neutral or slightly acid materials and in the case of cake dough a saturated sugar solution. Also that in dough any carbon dioxide generated by the baking powder and retained in the dough will leaven whereas in distilled water solutions the carbon dioxide generated must be completely driven off or it will reflect in the residual and yield a lower available carbon dioxide.

The amount of carbon dioxide retained in the residual solution is dependent on the following factors:

1. *p*H of the residual salts.
2. Amount and degree of gelatinization of the starch in the residual solution.
3. Concentration of soluble salts.

To obtain a true available CO_2 all the retained CO_2 which is in solution must be driven off. To my knowledge this can only be accomplished in one of two ways.

1. Evaporation to dryness.
2. By using a saturated solution of neutral sodium chloride or sodium sulphate as the reaction medium.

Evaporation to dryness is the basis of both of the Q.M.C. methods and it does bring the results down to lower levels which, in my opinion, are closer to the true values than the official method. Incidentally the modified Q.M.C. method is practically identical to the Official British method at present, the only difference being that the final determination of the CO_2 is done in an alkalimeter.

To those who think that evaporation to dryness is too severe, it may be pointed out that the use of a saturated sodium chloride solution instead of distilled water in the official A.O.A.C. method will bring the results down to the same level as those obtained with a single evaporation to dryness.

Gelatinization of the starch is another factor which makes it very difficult to drive out the available CO_2. Therefore some work should be done with the Q.M.C. method where evaporation to dryness is carried out at a temperature which is below the point at which starch gelatinizes. The gelatinized starch also causes trouble when the residual CO_2 is shaken out on the Chittick apparatus.

RECOMMENDATIONS*

It is recommended—

(1) That the present official method for determination of residual carbon dioxide in baking powders be dropped for the following reasons:

(a) The wording and description of the method are susceptible to too many interpretations.
(b) The manner of heating and treating the sample does not yield a true residual because all of the available CO_2 is not driven off.
(c) The method is not applicable to all types of baking powder, particularly the pyrophosphate types.

(2) That the Quartermaster Corps method (Rewritten Version) be adopted as a tentative method, adding the words "or electricity" after the phrase "The bath should be heated with gas."

(3) That the Q.M.C. method be subjected to further study wherein the single evaporation to dryness is carried out in a moisture oven instead of on a water bath and at temperatures ranging from 70 to 100°C.

(4) That the Q.M.C. method be also studied using a neutral saturated sodium chloride solution as a medium instead of distilled water.

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* For report of Subcommittee D and action by the Association, see *This Journal*, 31, 59 (1948).

ACKNOWLEDGMENT

The Associate Referee wishes to extend his thanks to all of the collaborators for their cooperation, and to Mr. Ludwig Dick (Standard Brands Inc., Chicago, Ill.) for his valuable suggestions and criticisms in this work.

No reports were given on disinfectants, or on leathers and tanning materials.

REPORT ON PLANTS

By ELROY J. MILLER (Michigan Agricultural
Experiment Station, East Lansing, Mich.) *Referee*

During the past year efforts have been made not only to continue the studies already in progress but to initiate new ones; however, not as much was accomplished in either respect as was originally hoped for.

Miss Lillian I. Butler, Associate Referee on Copper and Cobalt, submitted no report on either subject and asked to be relieved of this assignment.

Dr. J. T. Sullivan, Associate Referee on Carbohydrates, submitted no report on his subject. Sometime ago he asked to be relieved of this assignment, because the press of other duties prevented him from doing justice to it, and it is with regret that his request is acceded to at this time. The title of the subject has been changed to "Sugar."

Dr. J. S. McHargue, Associate Referee on Iodine and Boron, will present a report on boron but not on iodine.

Dr. E. J. Benne, Associate Referee on Carotene and Zinc, will report on the latter but not the former.

No report on sampling will be presented.

Steps are being taken to initiate studies on methods for determining the following constituents of plants: Cobalt, copper, fluorine, lead, sugar, starch, sodium, potassium, and different forms of nitrogen. A number of qualified analytical chemists have signified their willingness to serve as Associate Referees; however, the only definite assignment which has been made to date is that of Dorothy R. Waldron of the Michigan Agricultural Experiment Station, who has agreed to serve in this capacity for sodium. Other assignments will be made as soon as possible and when completed the Secretary will be notified of the appointees. It is hoped that the new appointments can be included in this report before it is published.

RECOMMENDATIONS*

It is recommended—

(1) That the recommendations made by the Associate Referees in their reports on Boron and Zinc, respectively, be accepted.

(2) That the following Associate Referees be appointed:

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* For the report of Subcommittee A and action by the Association, see *This Journal*, 31, 43 (1948).

Boron and Iodine—L. K. Wood, University of Kentucky, Agricultural Experiment Station, Lexington, Ky.

Carotene and Zinc—Erwin J. Benne, Dept. of Agricultural Chemistry, Mich. State College, East Lansing, Mich.

Cellulose and Lignin—Gordon H. Ellis, U. S. Plant, Soil, and Nutrition Laboratory, Ithaca, N. Y.

Cobalt and Copper—Kenneth C. Beeson, U. S. Plant, Soil, and Nutrition Laboratory, Ithaca, N. Y.

Pectin—C. O. Willets, Eastern Regional Research Laboratory, Chestnut Hill Station, Philadelphia, Pa.

Sampling—Elroy J. Miller, Department of Agricultural Chemistry, Mich. State College, East Lansing, Mich.

Sodium—Ray L. Shirley, Dept. of Agricultural Chemistry, Mich. State College, East Lansing, Mich.

Starch—Carroll L. Hoffpauir, Southern Regional Research Laboratory, New Orleans 19, La.

Sugar—Kenneth T. Williams, Western Regional Research Laboratory, Albany 6, Calif.

REPORT ON BORON IN PLANTS

By CALVIN M. AUSTIN and J. S. MCHARGUE (*Associate Referee*), Kentucky Agricultural Experiment Station, Lexington, Ky.

This report is an outline of a new colorimetric method for the determination of total boron in plant material. The reagent used is p-nitrobenzeneazo-1,8-dihydroxynaphthalene-3,6-disulfonic acid. This compound will be referred to as "Chromotrope-B." Feigl has reported the use of this compound as a spot test for boron.

The procedure is similar to the quinalizarin method, the chief difference being in the ignition of the sample. The ignition is made on air dry samples at 600°C. after the addition of an alkali.

Details of the method and the results obtained are described in a paper presented at this meeting, entitled "The Determination of Total Boron in Plant Material with 'Chromotrope-B'."[1]

PROCEDURE

Ignite 0.2 to 0.5 g of air-dried, 60-mesh sample in a platinum dish after the addition of barium hydroxide. The dish should be placed in a cool muffle furnace and the temperature raised to 600°C. The total time in the furnace should be two hours. After ignition extract the boron from the ash with 5.0 or 10.0 ml of 70% acetic acid. Add 10.0 ml of a .005% soln of "Chromotrope-B" in concentrated, sulfuric acid (reagent grade) to a 1.0 ml aliquot of the extract. Allow at least 30 min. for full color development and determine the per cent transmission spectrophotometrically at 620 mμ against a reference soln containing 1.0 ml of 70% acetic acid and 10.0 ml of the reagent soln. The boron content of the solution is then found from a standard curve determined by treating known amounts of boron in the same way.

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[1] Calvin M. Austin and J. D. McHargue, *This Journal*, page 427.

RESULTS

The results of analyses of several plant samples and a synthetic sample containing 97.2 p.p.m. of boron as anhydrous $Na_2B_4O_7$ are shown in Table 1.

TABLE 1.—*Results*

SAMPLE	BORON ADDED	NUMBER OF DETERMINATIONS	MEAN BORON FOUND	AVERAGE DEVIATION OF A SINGLE OBSERVATION	RECOVERY
	p.p.m.		*p.p.m.*	*p.p.m.*	*per cent*
Synthetic	97.2	9	92.7	1.1	95.4
Bean Seed		6	21.8	.4	
White Pine Needles (N)		5	38.0	1.1	
White Pine Needles (D)		5	42.6	1.1	
Wild Cherry Leaves (1)		4	17.1	.2	
Wild Cherry Leaves (2)		3	22.9	.4	
Kelp		6	314.3	2.8	

These results seem to justify further study. It is planned to continue this work particularly in regard to the analysis of biological material, soils, and fertilizers.

RECOMMENDATIONS*

It is recommended that the method be studied independently by others and that collaborative work be undertaken for next year.

REPORT ON ZINC IN PLANTS†

By RAY L. SHIRLEY, DOROTHY R. WALDRON, ELVA D. JONES, and ERWIN J. BENNE, *Associate Referee* (Michigan Agricultural Experiment Station, East Lansing, Mich.)

During the 1945 revision of *Methods of Analysis, A.O.A.C.* (1), the dithizone method for determining zinc in plant materials replaced the former H_2S-ferrocyanide procedure in the Plant Chapter. The dithizone method depends upon the combination of zinc with dithizone (diphenylthiocarbazone) in an ammoniacal solution of controlled reaction to form a red compound. This compound and some excess dithizone are removed from the aqueous solution by extracting it with carbon tetrachloride, and the zinc present is evaluated photometrically by a mixed-color procedure. Interference by copper is prevented by removing it as the dithizonate from acidified solution, and diethyldithiocarbamate is added to prevent cations other than zinc from forming dithizonates when the solution is made ammoniacal.

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* For report of Subcommittee A and action by the Association, see *This Journal*, 31, 43 (1948).

† Published with permission of the Director of the Agricultural Experiment Station as Journal article No. 957 (n.s.).

This method was published in its present form by Cowling and Miller (2). It was submitted for collaborative study by Cowling, who served as Associate Referee on zinc in plants from 1940 to 1941. Results from this study were reported at the meeting of the Association in 1940 and published in the Journal in 1941 (3). On the basis of these results Cowling recommended that the Association accept the method as a tentative one and that the study of it be continued. These recommendations were accepted; however, Cowling resigned as Associate Referee before acting upon his second recommendation.

The present Associate Referee was appointed in 1942 to succeed Cowling, but while the War was in progress other activities necessitated that an investigation of methods for determining zinc be postponed. During the past year, however, such an investigation was begun, and the results obtained to date are presented in this report.

EXPERIMENTAL AND RESULTS

It was decided to investigate the tentative A.O.A.C. method first, and later to study other procedures in an effort to find, or if possible to devise, one possessing additional advantages. To date the following aspects of the subject have been studied:

1. The spectral properties of carbon tetrachloride solutions of dithizone and zinc dithizonate, individually, and together as used in the mixed-color method.
2. The effects of daylight upon the spectral properties of these solutions and means of protecting them against such effects.
3. The following points in the mixed-color procedure:
    (a) Amount of dithizone reagent necessary when the concentration of zinc is high.
    (b) Standardization of the rinsing process with carbon tetrachloride after extraction of the aqueous solution with dithizone reagent.
    (c) Effects of varying the amount of solution B.
    (d) Effects of using a stock solution of carbamate reagent which was stored in a refrigerator.
4. (a) The practicability of removing excess dithizone from the carbon tetrachloride solutions of zinc dithizonate and using a one-color instead of a mixed-color procedure.
    (b) Determination of the accuracy with which known quantities of zinc added to extracts of plant ash, plus the zinc present in the extract, could be evaluated by use of this procedure.
5. Reproducibility of results by the mixed-color and one-color procedures when used by four different analysts in an intra-laboratory study.

Each of the above sections will be treated separately in the sequence given.

It was thought that a study of the spectral properties of the components of the mixed-color system should help to afford a clearer understanding of, and possibly lead to improvements in, the dithizone method. Consequently, spectral transmission curves were prepared, from 400 to 725 millimicrons, for carbon tetrachloride solutions of: (A) Dithizone reagent diluted 1 plus 4, (B) Zinc dithizonate and excess dithizone obtained by carrying 20 micrograms of zinc through the entire mixed-color procedure, and (C) Zinc dithizonate prepared by freeing a solution similar to (B) of excess dithizone with dilute aqueous ammonium hydroxide. A Cenco-Sheard Spectrophotelometer was used for this purpose. The results obtained are presented graphically in Figure 1.

H. R. Kraybill, one of Cowling's collaborators (3), called attention to the

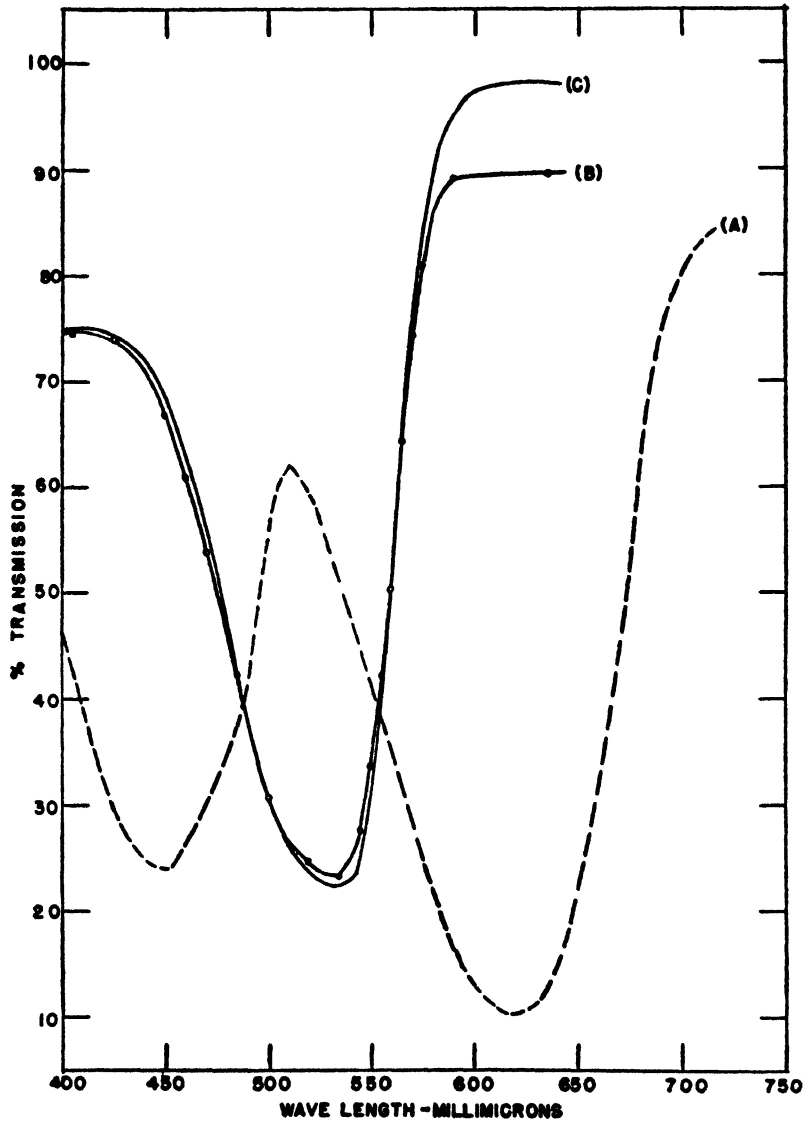

FIG. 1.—Spectral transmission curves of carbon tetrachloride solutions of (A) Dithizone reagent, (B) Zinc dithizonate and excess dithizone reagent, and (C) Zinc dithizonate.

tendency of the colors to fade when the carbon tetrachloride solutions of dithizone and zinc dithizonate involved in the mixed-color procedure were exposed to even ordinary diffused sunlight. He further observed that if electric light supplied the only illumination, fading did not occur, but rather the colors of the solutions tended to intensify during the first two hours under these conditions. When the solutions were maintained in darkness no changes in their color intensities were discernible in forty-eight hours.

During the early part of this investigation the authors corroborated Kraybill's observations. It occurred to them that the use of amber, or low-actinic, glassware might effectively protect the solutions against daylight. Accordingly separatory funnels, bottles, volumetric flasks, and pipettes made of such glass were obtained and used throughout the remainder of the investigation. Repeated trials showed this practice to be quite effective in stabilizing the optical densities of the carbon tetrachloride solutions. Given below are representative data showing the differences in the per cent of light transmitted, before and after a 3-hour exposure to daylight, by solutions of dithizone and zinc dithizonate which were carried through the mixed-color procedure, and maintained, in colorless or amber glassware:

KIND OF GLASSWARE USED	MICROGRAMS OF ZINC PRESENT	LIGHT TRANSMITTED	
		BEFORE EXPOSURE	AFTER EXPOSURE
		per cent	*per cent*
Colorless	0	67.0	82.0
Colorless	15	40.0	47.0
Colorless	20*	85.5	96.0
Amber	0	74.5	74.7
Amber	15	43.0	43.0
Amber	20*	87.3	87.5

* These solutions were diluted 10 times more than the others and per cent of light transmitted was determined with a Cenco-Sheard Spectrophotelometer In all other instances throughout the investigation a Cenco-Sheard-Sanford Photelometer, equipped with the light filter recommended by Cowling and Miller (2), was used for this purpose.

During extraction of the ash extract with dithizone reagent an excess of this reagent must be present, and the presence of an excess is indicated by an orange or yellow-orange coloration of the aqueous phase after the phases have separated. Such a color is difficult to see when separatory funnels made of amber or low actinic glass are being used; hence, another means of judging whether an excess was present became necessary. The authors found that the use of 15 ml. of dithizone reagent assured an excess for all of the plant materials which they sampled and analyzed according to the recommended procedure. Therefore, they adopted the practice of using this amount of reagent in all cases where the concentration of zinc was suspected of being high.

Likewise, in both the first and second extractions, rinsing with carbon tetrachloride is judged to be complete on the basis of the green coloration acquired by the carbon tetrachloride layer. Even with colorless glass vessels the authors found this to be a somewhat unreliable criterion, and with amber or low actinic glassware a revised rinsing technique was required. Repeated use showed the following procedure to be effective and reliable in both the first and second extractions: After making the first separation in the usual manner, add 2–3 ml. of carbon tetrachloride to the contents of the separatory funnel. Allow the layers to separate and draw off the lower layer; in the first extraction into the second separatory funnel; in the second into the container for waste carbon tetrachloride. Repeat once. Then add 3–5 ml. of carbon tetrachloride and shake vigorously for 15 seconds. Allow the layers to separate and draw off the lower layer as before. Repeat once.

Dithizone is very soluble in aqueous ammonia and presumably would be less readily extracted by carbon tetrachloride from solutions in which the concentration of ammonia is high. Hence, it was thought that differences in the amounts of solution B used might be partly responsible for variations in percentages of light transmitted by the carbon tetrachloride extracts. To determine if such were the case, varying amounts of solution B, and sufficient distilled water to make a total volume of 55 ml. were added to the usual quantities of 0.02 *N* HCl and dithizone reagent in each of three separatory funnels, and the remainder of the procedure was completed in the usual way. The volumes of solution B added and the percentages of light transmitted are given below:

Ml. of solution B added	*Per cent of light transmitted*
48	80.0
50	79.5
52	80.5

It is obvious from these results that significant variations in the percentages of light transmitted by the carbon tetrachloride extracts are not due to slight differences in the amounts of solution B used.

In an effort to eliminate variations in light transmission values that might accompany separate preparations of carbamate reagent for each series of determinations, a study was undertaken to ascertain how long a stock solution of this reagent could be stored and still be used safely. It was found that such a solution had no perceptible effect on the reproducibility of light transmission values for at least a month, if stored in the refrigerator when not in use. Hence, the practice of preparing a liter of this solution at a time, storing it in the refrigerator, and withdrawing required amounts immediately before use, was adopted.

It was thought that differences in the amount of dithizone removed from the aqueous phase in the final extraction might be responsible for the variations in the percentages of light transmitted by solutions containing the same amounts of zinc. If such were the case, it appeared that a one-color procedure, in which the excess dithizone was removed by extracting the carbon tetrachloride solutions with dilute ammonium hydroxide, should improve the reproducibility of transmission values. Cowling and Miller (2) discussed such a procedure, but they believed that when lead was present high values for zinc would result. However, they gave no data on this point; hence, it seemed of value to determine the effects of added lead. Accordingly similar solutions with and without lead were carried through the mixed-color procedure as usual. The aqueous layer was removed by use of suction, 50 ml. of 0.01 *N* NH_4OH were added to each separatory funnel, and the contents were shaken vigorously for 30 seconds. After the layers had separated, the carbon tetrachloride solutions were drawn off, aliquots were diluted as usual and the per cent of light transmitted by each was determined. Amounts of zinc and lead added and typical results obtained are summarized below:

Micrograms present		*Per cent of light*
Zn	*Pb*	*transmitted*
0	0	75.2
0	0	77.5
0	0	76.0
0	25	74.9
0	25	76.1
0	25	76.5
10	0	50.5
10	25	48.0
20	0	30.5
20	0	31.2
20	25	30.5
20	25	31.0

These data show no greater variations in results when added lead was present than when it was absent. Therefore, the procedure was tested further by applying it to the determination of zinc in extracts of plant ash.

A reference curve relating concentration of zinc and light transmission was prepared in the usual way by carrying known amounts of zinc through the entire procedure and determining the per cent of light transmitted by each carbon tetrachloride solution with the photoelectric colorimeter. Extracts of the ash from samples of plant materials were prepared as directed by the A.O.A.C. method, and aliquots were analyzed for zinc by the one-color procedure. Other aliquots were similarly analyzed after addition of known quantities of zinc. Representative results obtained, together with similar data pertaining to the mixed-color procedure reproduced from Cowling and Miller (2) for comparison, are given below.

Judged on the percentages of zinc recovered the two procedures appeared to be entirely comparable; hence, both were subjected to an intra-laboratory study participated in by four analysts. All were experienced analysts; however, analyst C had had no previous experience with

NATURE OF SAMPLE	ADDED[1]	MICROGRAMS OF ZINC		PER CENT OF TOTAL RECOVERED
		TOTAL PRESENT	DETERMINED[2]	
Lettuce leaves	0	—	11.88	100.0
	1.20	13.08	13.02	99.5
	2.40	14.28	13.85	97.0
Alfalfa leaf meal	0	—	8.25	100.0
	4.81	13.06	13.00	99.5
	9.62	17.87	17.58	98.4
	14.43	22.68	23.53	103.7
Cowling and Miller—				
Dried grapes	0	—	11.4	100.0
	10	21.4	21.9	102.3
	20	31.4	31.3	99.7
	40	51.4	51.2	99.6
Dried asparagus	0	—	13.6	100.0
	10	23.6	23.4	99.2
	20	33.6	33.5	99.7
	40	53.6	53.6	100.0

[1] Added to the lettuce, grape, and asparagus samples prior to ashing and to aliquots of the extract of ash from alfalfa meal immediately before analyzing.

[2] Averages of results from 2 or more determinations.

either procedure. Each participant prepared, and used, his own reference curves relating concentration of zinc and light transmission. The data from which these curves were constructed are shown below as an indication of the comparative reproducibility of results with known quantities of zinc when the two procedures were used by different analysts.

MICRO-GRAMS OF ZINC	PER CENT OF LIGHT TRANSMITTED							
	MIXED-COLOR PROCEDURE, ANALYST—				ONE-COLOR PROCEDURE, ANALYST—			
	A	B	C	D	A	B	C	D
0	74.5	70.0	71.2	72.1	83.0	81.0	79.1	79.8
10	51.6	48.0	48.7	47.6	50.0	48.1	48.8	48.5
15	41.5	39.9	42.2	40.6	39.0	36.9	—	38.0
20	33.9	32.1	32.0	33.7	31.9	30.1	30.1	30.0
25	28.9	28.0	27.1	29.7	26.0	24.1	25.2	24.0
30	24.0	23.1	23.3	25.0	19.9	19.3	21.0	20.0

Fortunately it was possible to obtain for this study portions of the three samples analyzed by Cowling and his collaborators (3). Extracts of each were prepared, and all four participants analyzed aliquots by both procedures. Results obtained, together with those obtained by Cowling and his collaborators with the mixed-color procedure (3) to permit comparison of reproducibility, follow:

ANALYST	P.P.M. OF ZINC DETERMINED[1]					
	MIXED-COLOR PROCEDURE SAMPLE NO.[2]			ONE-COLOR PROCEDURE SAMPLE NO.[2]		
	1	2	3	1	2	3
A	27.8	23.4	46.6	22.4	24.1	46.4
B	27.4	24.1	45.0	22.5	21.9	45.3
C	25.5	19.8	42.7	23.1	24.1	46.7
D	26.7	21.5	46.2	22.0	22.9	46.5
Av.	26.9	22.2	45.1	22.5	23.3	46.2
Cowling's collaborators—						
No. 1	26.4	22.3	49.3			
No. 2	24.6	19.7	49.5			
No. 3	21.0	17.8	45.8			
No. 4	26.5	23.5	42.5			
Cowling—	25.3	22.3	46.9			
Av.	24.8	21.1	46.8			

[1] Averages of results from 2 or more determinations.
[2] Sample 1 was alfalfa leaf meal, 2 was parsnip roots, and 3 was spinach leaves.

Agreement of results by the two procedures when used by different analysts was quite satisfactory; moreover, the values for zinc agreed well with those obtained by Cowling and his collaborators. Unfortunately, the extracts used with the mixed-color procedure became exhausted, and others had to be prepared for use with the one-color procedure. Hence, errors attendant with the ashing and extracting processes are probably reflected in the differences in results obtained for a given sample by the two procedures. This appears to be especially true of sample 1.

DISCUSSION

Cowling and Miller (2) published a spectral transmission curve for a carbon tetrachloride solution of zinc dithizonate. However, they gave none for dithizone alone nor for a mixture of dithizone and zinc dithizonate as used in the mixed color procedure; therefore, the curves shown in Figure 1 appeared to be of sufficient interest to include in this report.

Curve (C) for zinc dithizonate is practically identical with that of Cowling and Miller. It corroborates their choice of a light filter, which transmitted light most strongly near 535 millimicrons, for use in a photoelectric colorimeter. The authors used a wave length setting of 530 millicrons on a Cenco-Sheard Spectrophotelometer and obtained excellent reproducibility of results with carbon tetrachloride solutions of zinc dithizonate of the same concentration.

It will be noted that curve (A) for dithizone reagent exhibits two minima and one maximum. Fortunately the latter coincides closely with the region of strong absorption by zinc dithizonate. This shows that by taking transmission readings in this region for evaluating the concentration of zinc dithizonate, much of the effect of excess dithizone is eliminated. Curve (B) for a mixture of dithizone and zinc dithizonate further illustrates this point. It shows that the excess dithizone caused only slight digression from curve (C) except in the region of strongest absorption, and should have, therefore, but little effect on readings taken between 530 and 540 millimicrons.

This investigation yielded the following points of information which should be helpful in the determination of zinc as the dithizonate:

1. Unless daylight can be effectively excluded, use of amber or low actinic glassware should aid in obtaining reliable results.

2. The procedure described for rinsing the aqueous solutions with carbon tetrachloride following extraction with dithizone reagent is more convenient and reliable than that now included in the tentative method.

3. Withdrawal of required amounts of carbamate reagent from a properly stored stock solution immediately before use is a more convenient and efficient practice than preparing a fresh solution of this reagent each time the procedure is used.

4. The intra-laboratory study conducted by the authors showed that the results obtained with the one-color procedure by different analysts were as consistent as those obtained for the same samples with the mixed-color procedure. The one-color procedure gave somewhat more consistent values for blank corrections and low concentrations of zinc than it was possible to obtain with the mixed-color procedure. It should be pointed out, however, that the phasic separations common to both procedures appear to be responsible for experimental variations which could not be entirely overcome by exercise of the most rigid care in manipulation.

It is recommended* that the study be continued.

LITERATURE CITED

(1) *Methods of Analysis, A.O.A.C.*, 6th Edition, 1945.
(2) *Ind. Eng. Chem., Anal. Ed.*, **13**: 145 (1941).
(3) *This Journal*, **24**: 520 (1941).

No report was given on sampling, iodine, carbohydrates, copper and cobalt, or carotene, in plants.

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* For report of Subcommittee A and action by the Association, see *This Journal*, **31**, 43 (1948).

MONDAY—AFTERNOON SESSION

REPORT ON PROCESSED VEGETABLE PRODUCTS

By V. B. **Bonney** (Food and Drug Administration, Federal Security Agency, Washington D. C.), *Referee*

RECOMMENDATIONS*

It is recommended—

(1) That studies of methods for quality be continued.

(2) That studies be continued on determination of moisture in dried vegetables.

(3) That studies for methods for determination of enzymes in frozen vegetables be continued.

No reports were given on quality factors, moisture in dried vegetables, or catalase in frozen vegetables.

No reports were given on fill of container methods, or on coffee and tea.

REPORT ON COLORING MATTERS IN FOODS

By C. F. **Jablonski** (Food and Drug Administration, Federal Security Agency, New York 14, N. Y.), *Referee*

The Committee of the A.O.A.C. requested the Referee to continue the collaborative study for the detection of small amounts of tartrazine FD&C Yellow #5 in alimentary paste products. The method proposed (published in the "Method of Anlaysis" A.O.A.C., 6th Ed. Chapter 20, 125, page 268) has already been studied collaboratively; however, a slight modification was deemed essential in order to insure better results. With this consideration in view, the Referee sent out a number of alimentary paste samples of five (5) subdivisions each to various collaborators, with instructions to follow the method as published, but use only 0.1 ml. of saturated aqueous hydrazine sulfate solution, instead of the stated 1.0 ml (third line from bottom of paragraph). The composition of the samples was as follows:

No. 1, *Egg Noodles* containing 3 p.p.m. of FD&C Yellow No. 5
No. 2, *Egg Noodles*, free from any added color
No. 3, *Macaroni*, containing 3 p.p.m. of FD&C Yellow No. 5
No. 4, *Macaroni*, free from any added color
No. 5, *Macaroni* containing 2 p.p.m. of FD&C Yellow No. 5

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* For the report of Subcommittee C and action by the Association, see *This Journal*, 31, 49 (1948).

The report of the collaborators is as follows:

N. E. Freeman Atlanta Station	Sample No. 1—color—present 2—color—absent 3—color—present (strong) 4—color—doubtful 5—color—present
W. S. Cox Atlanta Station	Sample No. 1—color—present 2—color—absent 3—color—present (strong) 4—color—absent 5—color—present
M. C. Harrigan (Miss) Boston Station	Sample No. 1—color—positive 2—color—negative 3—color—positive less than in 1 4—color—negative 5—color—positive less than in 3
M. Matluck Boston Station	Sample No. 1—color—positive 2—color—negative 3—color—positive 4—color—negative 5—color—positive
C. S. Purcell Boston Station	Sample No. 1—color—positive 2—color—negative 3—color—positive 4—color—negative 5—color—positive
A. L. Suslam Boston Station	Sample No. 1—color—positive 2—color—negative 3—color—positive 4—color—negative 5—color—doubtfully positive
H. I. Macomber Baltimore Station	Sample No. 1—color—positive 2—color—negative 3—color—faintly positive 4—color—negative 5—color—positive
S. M. Walden Baltimore Station	Sample No. 1—color—positive 2—color—negative 3—color—positive (faint) 4—color—negative 5—color—positive
J. L. Hogan New York Station	Sample No. 1—color—present 2—color—absent 3—color—present 4—color—absent 5—color—present

M. L. Offutt (Miss)	Sample No. 1—color—positive
New York Station	2—color—negative
	3—color—positive
	4—color—negative
	5—color—positive
F. C. Minsker	Sample No. 1—color—positive
Philadelphia Station	2—color—negative
	3—color—positive
	4—color—negative
	5—color—positive
H. M. Boggs	Sample No. 1—color—positive
Philadelphia Station	2—color—negative
	3—color—positive
	4—color—negative
	5—color—positive
R. T. Marwin	Sample No. 1—color—positive
Conn. Agricultural Experiment	2—color—negative
	3—color—positive
	4—color—negative
	5—color—positive
J. J. Winston	Sample No. 1—color—positive
Jacobs Cereal Products Labor,	2—color—negative
New York	3—color—positive
	4—color—negative
	5—color—positive
J. M. Bisogno (Miss)	Sample No. 1—color—positive
Jacobs Cereal Products Labor,	2—color—negative
New York	3—color—positive
	4—color—negative
	5—color—positive

COMMENTS BY THE COLLABORATORS

N. E. Freeman: Filtration was slow but clear and no precipitate was formed in the coupling test.

W. S. Cox: No difficulties were encountered in the coupling test.

M. C. Harrigan: Suggest a pancreatic digestion of the alimentary paste for larger recovery of color.

M. Matluck: Samples 1, 3, and 5 gave a definite yellow color on wool, whereas samples 2 and 4 gave only a straw color. However, the usual spotting reagents gave no clear differentiation between positive and negative samples.

C. S. Purcell: No comments.

A. L. Suslam: No comments.

H. I. Macomber: No comments.

S. M. Walden: In general the dyeing tests were all less conclusive than the coupling. None of the solutions were clear after the additions of the precipitants with subsequent centrifuging.

J. L. Hogan: I experienced no particular difficulties with those determinations. In samples 1, 3, and 5 the pieces of wool took on a decided yellow color, while in 2 and 4 the cloth remained almost colorless. In the former cases, the spot tests resembled those of standard tartrazine of similar intensity. While the color produced by the coupling test is not very deep yet there seems to be sufficient color developed to differentiate these from the uncolored samples.

M. L. Offutt: Coupling test very definite in showing the presence of color. Method worked well throughout.

F. C. Minsker: The method as described was used and very good results obtained.

H. M. Boggs: Some trouble with the coupling was experienced on the first attempt, but definite though fairly light pinks were produced in the second attempt.

R. T. Marwin: No comments.

J. J. Winston: Samples 1 and 2 were examined twice by the recommended procedure with a slight modification the second time. After the protein matter was precipitated by the specified reagents, the flask was placed overnight in the refrigerator. The clear solution was then further examined according to the prescribed procedure and the results indicated that more of the protein matter was removed due to freezing This resulted in a better dyeing and coupling test for sample #1.

J. M. Bisogno: There was little difficulty in detecting the added color. The coupling reactions particularly were very good.

DISCUSSION

The results reported by the collaborators indicate that by the application of the method as published in the *Methods of Analysis*, 6th Ed., with the incorporation of a slight change, the presence of very small amounts of tartrazine can definitely be established. It is gratifying to state that every one of the coworkers has been able to detect the added coloring matter, although the majority were probably unfamiliar with the method. Some collaborators report more or less cloudiness of the solution preparatory to dyeing. It is the Referee's observation that this condition which has been generally prevalent during the warm season will in no way hinder the dyeing and stripping operation. However, if time permits, it is advantageous to place the solution in the refrigerator overnight for complete clarification. This suggestion has been offered by collaborator W.

To sum up results: Tartrazine FD&C Yellow No. 5 can be detected positively by the use of the described method in 200 gm. of material containing as low as 2 p.p.m. of dye.

The Referee desires further to report considerable progress in the problem of a quantitative separation and estimation of Tartrazine FD&C Yellow No. 5 and Sunset Yellow FCF, FD&C Yellow No. 6.

A method for distinguishing between Oil Orange SS, FD&C Orange No. 2, and Oil Red XO, FD&C Red No. 32 was studied.

Oil Orange SS and Oil Red XO are both oil soluble azo dyes, differing in their respective chemical structure by only a methyl group. Both dyes respond similarly towards acids, alkalies, and reducing agents. The dyed fabric is also of similar shade. It was, therefore, impossible up to now to distinguish between these colors in the usual manner. However, experiments disclosed that under certain definite conditions Orange SS is precipitated as a brick red flaky precipitate, while Oil Red XO under similar condition remains in solution.

The mode of procedure is as follows: Solutions required—formaldehyde 39%; pyridine, U.S.P.

Prepare a dye solution of approximately 0.001% in ethanol.

Color solution 0.001%	2 ml.
Water	16 ml.
Pyridine	0.15 ml.
Formaldehyde	0.20 ml.

Place in the order named above into a 50 ml Erlenmeyer flask. In presence of Orange SS the solution becomes slightly opalescent in approximately 5 minutes and later (in about 30 minutes) a brick red precipitate is apparent. Oil Red XO treated similarly remains in orange solution.

In presence of both dyes (in above concentration) the precipitation of Orange SS will not be apparent before 12 or more hours, depending upon the percentage of Oil Orange SS present in the mixture. However, if the dye concentration should be 0.005% or over the precipitation of Orange SS takes place immediately, but Oil Red XO will also partially precipitate after approximately 30 minutes. It is, therefore, desirable to keep the color concentration between 0.001–0.002%.

RECOMMENDATIONS*

The Referee therefore recommends—

(1) That the rapid method of detection of small amounts of Tartrazine FD&C yellow No. 5 (as published in the *Book of Methods,* VI) with the proposed slight modification be made official (final action).

(2) That investigational work be continued on the quantitative separation of FD&C Yellow No. 5 (Tartrazine) and FD&C Yellow No. 6 (Sunset Yellow FCF).

(3) That investigational work be undertaken to separate and determine quantitatively FD&C Green No. 2 (Light Green SF Yellowish), FD&C Green No. 3 (Fast Green FCF), and FD&C Blue No. 1 (Brilliant Blue FCF).

(4) That investigational work be undertaken to separate and estimate quantitatively FD&C Yellow No. 3 (Yellow AB), FD&C Yellow No. 4 (Yellow OB), FD&C Orange 2 (Orange SS), and FD&C Red No. 32 (Oil Red XO).

(5) That collaborative work on analytical methods for coal-tar colors certifiable for use in foods be conducted.

REPORT ON DAIRY PRODUCTS

By GUY G. FRARY (State Chemical Laboratory,
Vermillion, S. Dak.), *Referee*

The following recommendations† are made as Referee on Dairy Products.

(1) That the Sander-Sager phosphatase test, as reported by the Associate Referee, be made official, first action, for detection of incomplete pasteurization of fluid milk and cream, cheddar type cheeses, and soft unripened cheeses.

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* For report of Subcommittee C and action by the Association, see *This Journal,* 31, 49 (1948).

† For report of Committee C and action by the Association, see *This Journal,* 31, 50 (1948).

(2) That the Sanders-Sager phosphatase test, as reported by the Associate Referee, be adopted as tentative for detection of incomplete pasteurization of types of cheeses other than those named in (1), ice cream mix, sherbet mix, milk drinks, butter, buttermilks, goats' milk, cheese whey, and concentrated milk products; and that the method be studied collaboratively during the coming year.

(3) That the methods for phosphatase tests set out in **22.43–22.57**, inclusive, be dropped.

(4) That the rapid method for moisture in cheese, employing the higher temperature of 130°, be adopted as tentative and that study of methods for sampling, fat, and moisture in cheese be continued.

(5) That the method of preparation of butter samples by means of mechanical stirring be adopted as official, first action.

(6) That the present official method for preparation of butter samples be reworded as recommended, with some condensation.

(7) That the present tentative method for mechanical preparation of butter samples be dropped.

(8) That application of shaking machines in the official method be studied collaboratively under one familiar with such application.

(9) That studies be continued on methods for detection of reconstituted milk.

(10) That studies be continued on ash in milk and in evaporated milk.

(11) That studies be continued on chlorine in milk.

(12) That studies with collaborative work be made of the method for determination of the acidity of milk.

(13) That an associate referee on fat in homogenized milk be appointed and studies be undertaken to develop an accurate method.

(14) That the method for fat in ice cream (**22.149**) be editorially changed by adding, after "60°" at the end of the second sentence, the following: "for 20 min. with occasional shaking"; and by changing the third sentence in the method to read as follows: "cool and proceed as directed under **22.25**, beginning with 'Add 10 ml alcohol and mix well.'"

(15) That statements be incorporated in the methods for added water in milk which employ the Zeiss immersion refractometer, which will give the comparable readings obtained under the same conditions by the Bausch and Lomb instrument: (**22.28 (a)**, **22.29**, and **22.30**).

(16) That study of the serum methods for added water in milk (**22.28**, **22.29**, and **22.30**) be continued.

(17) That the sediment test **22.40**, **22.41**, and **22.42**, be dropped, and that the A.P.H.A. method be adopted as tentative.[1]

(18) That the cryoscopic method for added water in milk, **22.33**, (p. 316) be editorially corrected by inserting the word "depression" after "point" line 6, p. 316; and by deleting "depression" in line 9 and in 3rd line from end of method, on the same page.

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[1] The details of the method are given in *This Journal*, **31**, 93 (1948).

REPORT ON SAMPLING, FAT, AND MOISTURE IN CHEESE

By WILLIAM HORWITZ (*Associate Referee*) and LILA KNUDSEN (U. S. Food and Drug Administration, Minneapolis, 1, Minnesota, and Washington 25, D. C.)

The previous report of the Associate Referee[1] presented an outline of an experiment designed to determine the moisture distribution in small cheeses of the Cheddar variety. Eight cheeses in this series have been analyzed for moisture; more work on cheeses of other sizes is contemplated.

The modified fat method presented in the previous report, has been subjected to collaborative study. Three types of cheeses were used in this work: a process American, a rindless Cheddar, and a daisy Cheddar. The cheese was frozen[2] at 0°F and a portion from each cheese was shredded while still frozen.[3] About one quart of the shredded material was placed in a two quart jar and the contents were thoroughly mixed by rolling and shaking. The shredded samples were then allowed to stand at 0°F for about a week for "tempering" and they were then remixed. Small portions from the two quart jars were transferred to ten four ounce screw cap "cheese jars" in rotation until all of the original material was distributed. In this way some of the top, middle, and bottom portions of the cheese in the original container was present in each of the small sample jars. The small samples were mixed, capped, and packed with dry ice for shipment. Each collaborator received two jars of cheese from each of the three cheeses. The first five jars from the process American cheese were given the sample number 1; the last five jars, the sample number 2. The first five jars from the rindless Cheddar were given the sample number 3; the last five jars, the sample number 4. The first five jars from the daisy Cheddar were given the sample number 5; the last five jars, the sample number 6. The first collaborator received the first and sixth jar from each cheese; the second, the second and seventh; etc. Thus each of the five collaborators received six samples, each consecutive set of two numbers being duplicates. Duplicate samples were sent to the collaborators rather than requesting them to run duplicates from a single sample in order to obtain information regarding the homogeneity of the original shredded material.

The collaborators were instructed in detail regarding further remixing of the samples before analysis and were given a definite order for removing portions for analysis so that manipulative details up to the point of weighing the analytical portions would be fairly consistent among the analysts. Four determinations were performed on each sample: Moisture by two methods—I, the official method, and II, 1.25 hours in the forced draft oven at 130°C; and fat by two methods—III, direct weighing of sample

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[1] *This Journal*, 30, 421 (1947).
[2] This temperature was lower than necessary but a 20–30°F. cold room was not available.
[3] The Grisoer Grater (Grisoer Industries, Fort Wayne, Ind.) was used for shredding.

TABLE 1.—*Individual results on collaborative cheese samples*

(Results in parentheses are reruns and are not included in the calculations)

COLLAB-ORATOR	SAMPLE	MOISTURE		FAT		FAT D.B.†	
		METHOD I	II	III	IV	III	IV
A	1	39.19	39.08	31.24	31.02	51.37	51.01
	2	38.91	39.13	31.15	30.90	50.99	50.58
B	1	39.04	39.28	30.83	30.89	50.57	50.67
	2	39.08	39.11	30.99	30.87	50.87	50.67
C	1	39.02	39.08	31.08	31.22	50.97	51.20
	2	38.96	38.77	30.86	31.01	50.56	50.80
	1	(38.79)	(38.71)				
	2	(39.01)	(39.03)				
D*	1	39.02	38.78	30.53	30.10	50.07	49.36
	2	39.29	38.98	29.86	29.92	49.18	49.28
E	1	39.02	39.08	31.11	31.37	51.02	51.44
	2	38.86	39.28	31.24	31.25	51.10	51.11
A	3	36.03	36.14	35.27	35.20	55.14	55.03
	4	35.99	36.48	35.26	35.24	55.08	55.05
	3		(36.59)				
	4		(36.31)				
B	3	36.04	36.29	35.08	34.96	54.85	54.66
	4	36.04	36.29	34.82	34.89	54.44	54.55
C	3	35.74	35.80	35.35	35.25	55.01	54.86
	4	35.58	36.07	35.29	35.12	54.78	54.42
	3	(35.41)	(35.43)				
	4	(35.52)	(35.49)				
D*	3	36.34	36.18	34.32	34.12	53.91	53.60
	4	36.18	36.15	33.92	33.96	53.15	53.21
E	3	36.09	36.37	35.40	35.30	55.39	55.23
	4	36.25	36.33	35.36	35.19	55.47	55.20
A	5	36.40	36.66	32.35	32.18	50.86	50.60
	6	36.43	36.82	32.38	32.14	50.94	50.55
	5	(36.71)					
B	5	36.72	37.04	31.91	31.86	50.43	50.35
	6	36.69	36.80	31.95	31.77	50.47	50.18
C	5	37.02	36.49	32.40	32.13	51.44	51.02
	6	36.00	35.98	32.06	32.23	50.09	50.36
	5	(35.70)	(35.71)				
	6	(36.04)	(36.65)				
D*	5	36.88	36.51	31.28	30.90	49.56	48.95
	6	36.86	36.56	31.69	31.35	50.19	49.65
E	5	36.37	36.75	32.29	32.15	50.75	50.53
	6	36.46	36.77	32.39	32.45	50.98	51.07
	5				(32.33)		(50.81)

* Apparently a constant error is present in the fat of this collaborator's determinations.
† Calculated on results for moisture by Method (I).

into and digestion in the Mojonnier tube; and IV, the official method. The methods used are as follows:

MOISTURE

I. *Official Method:* Sec. 22.124.

II. *Modified Method:* Weigh 2–3 g of prepared sample into moisture dishes with tight-fitting covers. Partially dry on the steam bath with lids removed and then insert in a forced draft oven which has come to equilibrium at 130 ± 1°C. Dry for 1.25 hours (with covers entirely off), cover tightly, remove from oven, cool, and weigh.

FAT

III. *Modified Method:* Weigh 1 g prepared sample into a tared (with stopper) Mojonnier tube using a rounded blade spatula. Introduce the cheese so that no particles are left on the neck of the tube and so that the cheese is deposited as close to the lower neck as possible. Stopper and reweigh. Shake the particles into the bulb with a sharp tap on the palm of the hand. Add 10 ml NH_4OH (1+9) from a pipet, washing down the sides of the tube. Place in a hot water bath (70–80°C) and shake frequently until the casein is well softened. Cool slightly, neutralize with HCl using litmus as indicator, and add 10 ml more of HCl. Mix and heat in a boiling water bath until the casein is digested and the liquid has darkened (15–40 minutes). Shake occasionally to wash down any particles which may have remained in the upper part of the tube. When digestion is complete, cool and extract fat according to 22.130.

IV. *Official Method:* Sec. 22.130. Report moisture, fat, and fat on the dry basis (using moisture by the official method).

RESULTS AND DISCUSSION

Table 1 presents the individual results obtained by the collaborators. Results in parentheses are reruns which are not included in the calculations of averages and standard deviations. Table 2 summarizes the data and compares it with the averages and standard deviations found by Ferris[4] in his comparison of the official fat method with a method similar to that above except for the use of a special "Ferris tube" instead of the Mojonnier tube. The data are also compared with the results of Lepper and Hart[5] on jar samples of cheese run for moisture and fat by the official methods.

An analysis of the collaborative results can be best shown graphically. Since primary interest lies in the variation from sample to sample and the variation between laboratories, we must use some method of putting the results from the three types of cheeses on the same basis so that they may be compared. Accordingly, a general average or mean was calculated for all results by the official A.O.A.C. moisture method on samples 1 and 2, and differences were obtained between each result and that general average. The same thing was done for samples 3 and 4, and then for samples 5 and 6. Since these differences are all of the same relative magnitude, they can be plotted together on the same scale and grouped by collaborator as shown on the left hand side of Figure 1 for the official moisture method. The right hand side of Figure 1 shows the same thing for the modified moisture method; and Figure 2 treats similarly the modified method for fat and the official method for fat.

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[4] L. W. Ferris, U. S. Food and Drug Administration, Buffalo, New York, unpublished study.
[5] H. A. Lepper and L. Hart, *This Journal*, 16, 584 (1933), Table 2.

TABLE 2.—*Summary of averages and standard deviations of collaborative cheese samples in this and previous studies*

This study: 5 collaborators, 10 determinations

	PROCESS NO. 1 & NO. 2	RINDLESS NO. 3 & NO. 4	CHEDDAR NO. 5 & NO. 6	AVERAGE STANDARD DEVIATION FOR METHODS*
Moisture I—Official:				
Average	39.04	36.03	36.58	
Std. Deviation	.13	.23	.31	.23
Moisture II—130°, 1.25 hours:				
Average	39.06	36.21	36.64	
Std. Deviation	.17	.19	.28	.22
Fat III—Modified:				
Average	30.89	35.01	32.07	
Std. Deviation	.42	.51	.37	.44
Fat IV—Official:				
Average	30.86	34.92	31.92	
Std. Deviation	.48	.48	.47	.48

Results reported by Ferris.[4]

	Sample 1		*Sample 2*	
	Official	*Ferris Tube*	*Official*	*Ferris Tube*
Fat, Average	27.69 (7, 16)†	27.73 (7, 14)	28.89 (5, 11)	28.88 (5, 10)
Std. Deviation	0.43	0.40	0.33	0.35

Results reported by Lepper and Hart.[5] (Table 2, Jar Samples)

Moisture, Average	36.22 (11, 27)†	Fat, Average	30.37 (11, 28)
Std. Deviation	0.25	Std. Deviation	0.40

* Obtained by taking the square root of the average of sums of squares of individual standard deviations.

† Figures in parentheses denote, respectively, the number of collaborators and total number of determinations.

From two figures following it can be seen that the variation between the results from samples of the same cheese at one laboratory is negligible, compared to the variation from one laboratory to another. This indicates a homogeneous shredded sample of each cheese, and that any difference between results of different collaborators is not due to a difference between the samples sent to them. This has also been shown by a statistical technique called analysis of variance, from which were obtained the two types

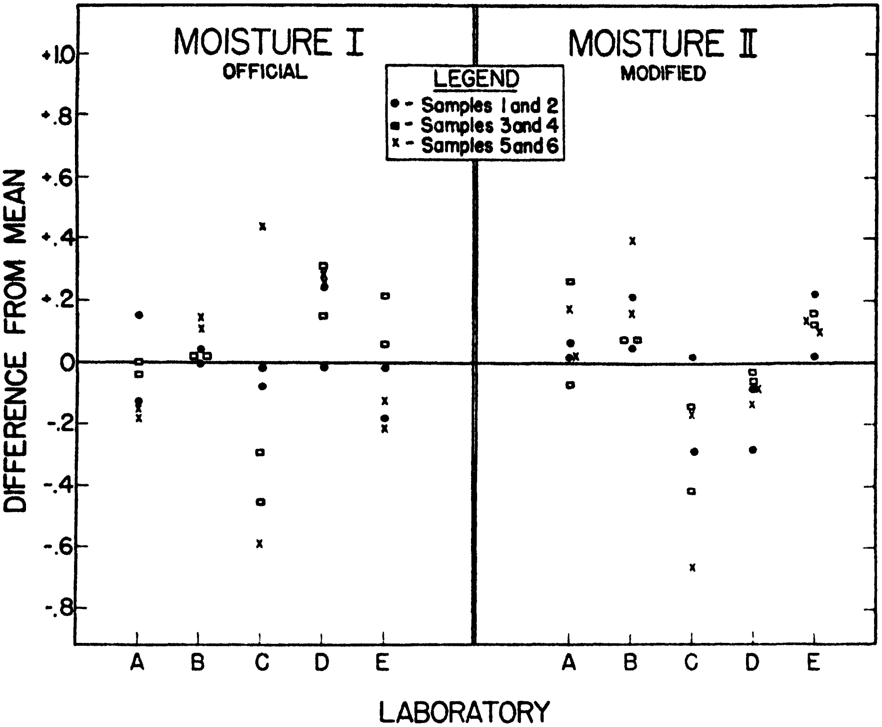

FIG. 1.—Differences of individual moisture determinations from the average determination for each cheese.

For moisture methods I and II, the over-all standard deviations are 0.23 and 0.22, respectively, and the standard deviations within stations (average standard deviation for any one station) are 0.21 and 0.16, respectively.

of standard deviations shown on each figure—the over-all standard deviation on a particular method and the standard deviation within the stations. These two standard deviations indicate respectively how closely the method can be checked at any laboratory, and how closely it can be checked at the same laboratory.

An interesting observation can be made from the charts; each collaborator's results have about the same position on both methods for each constituent, for instance, collaborator C is low on both moisture methods; collaborator B is high on both moisture methods; collaborator B is average on both fat methods; collaborator D is low on both, etc.

The over-all standard deviations of the official moisture and the modified moisture method are not significantly different (.231 and .222, respectively). The same is true of the standard deviations for the modified fat method and the official fat method (.437 and .477, respectively). These are about the same as those found by Ferris[4] and Lepper and Hart.[5] The average moisture by the modified method is slightly higher than that obtained by the official method. The difference, about 0.1 per cent, is so small that the modified method can be used as a rapid screening moisture test. It is therefore recommended as such. The average fat by the modified method is slightly higher, by about 0.1 per cent, than by the official method. This may be attributed to the elimination of the fat transfer from beaker to flask which is required in the official method, as well as to the impossibility of fat loss from spattering in the modified method. As one collaborator commented, "The uncertainty in the official method due to loss in boiling and possibly in transferring makes method III a desirable improvement."

Practically all of the collaborators performed this work in hot, humid weather. One mentioned that moisture condensed on the spatula during the preliminary mixing, but when these samples were rerun the moisture values were usually lower than those obtained on the original determination. It would appear desirable to repeat this collaborative work under more favorable conditions in the fall or early winter and to extend it to other varieties of cheese.

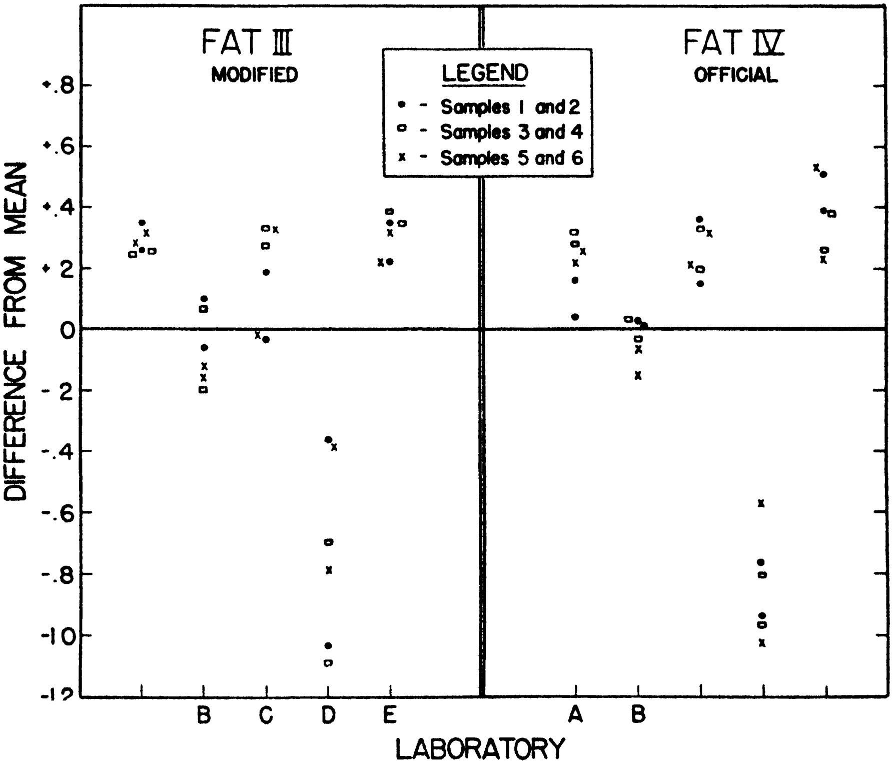

FIG. 2.—Differences of individual fat determinations from the average determination for each cheese.

For fat methods III and IV the over-all standard deviations are 0.44 and 0.48, respectively, and the standard deviations within stations (average standard deviation for any one station) are 0.19 and 0.13, respectively.

Three of the five collaborators reported loss of some fat determinations by the official method because of violent bumping. From the limited experience of the associate referee, it would appear that the required use of sand in the official fat method actually favors bumping rather than diminishes it. The statement in this method ". . . neutralize with HCl, using litmus as indicator" is ambiguous. Should litmus in the form of solid, solution, or paper be used? We may even go further and inquire whether such a strict neutralization is required or whether it can not be assumed that 1 ml of HCl (0.012 moles) is roughly equivalent to 1 ml of NH_4OH (0.015 moles) especially when a ten-fold excess of HCl is finally present.

SOFT CHEESES

It has been brought to the attention of the Associate Referee that the present official method for the preparation of cheese samples (**22.123**) is not applicable to the soft cheeses. Preparation of samples of cottage, creamed cottage, and cream cheeses is important both for composition and for phosphatase determinations. Such a study will be inaugurated next year.

ACKNOWLEDGMENT

Grateful acknowledgment is due to the following collaborators (all of the U. S. Food and Drug Administration): Sidney Williams, Minneapolis; John H. Bornmann, Chicago; F. J. McNall, Cincinnati; and N. Aubrey Carson, St. Louis.

RECOMMENDATIONS*

It is recommended—

(1) That the forced draft oven method for moisture in cheese be adopted as tentative.

(2) That study of methods for sampling, fat, and moisture in cheese be continued.

(3) That studies of methods for preparation of samples of soft cheeses be undertaken.

No report was given on ash in milk and evaporated milk.

REPORT ON THE PHOSPHATASE TEST IN PASTEURIZATION OF DAIRY PRODUCTS

By George P. Sanders (Division of Dairy Research Laboratories,
Bureau of Dairy Industry, Agricultural Research Administration,
Department of Agriculture, Washington 25, D. C.),
Associate Referee

A description of the Sanders-Sager modification of the phosphatase test applied to Cheddar cheese and fluid milk was published in 1946 (1). The modified test was developed as a result of urgent requests from members of the cheese industry and public health officials to investigate the possibility of developing a reliable method for testing cheese to determine accurately whether or not the milk used in making the cheese had been adequately pasteurized. The details of the method proposed are the result of exhaustive research on available tests for pasteurization and on the chemistry of the milk phosphatase enzyme and the quantitative measurement of its activity.

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* For report of Committee C and action of the Association, see *This Journal*, 31, 50 (1948).

While the research work was under way and in process of publication, collaborative work was done by Dr. F. W. Gilcreas, Associate Referee on the phosphatase test for hard cheese, and by William Horwitz, Associate Referee on the phosphatase test for soft cheese. In Dr. Gilcreas' work (2), both a modified Kay-Graham and the Sanders-Sager methods were investigated collaboratively, with tests on a large number of samples. The results showed, as Sanders and Sager also had found, that the modified Kay-Graham procedure could not be adapted satisfactorily as an index of pasteurization in testing cheese. Gilcreas found that the Sanders-Sager method provides a reliable estimate of the inactivation of the enzyme by heat, and thus of the degree of heat treatment of the milk from which hard cheese was made. He recommended that it be adopted as a tentative method.

Horwitz (3) likewise found that the Sanders-Sager method could be applied successfully in testing soft cheeses. Further, he referred to the possibility of applying to dairy products the method proposed for blood serum by Bessey *et al.* (4), in which *p*-nitrophenyl phosphate (colorless) is used as the substrate and the enzyme hydrolyzes it to *p*-nitrophenol (yellow); he suggested that this procedure be investigated.

During the progress of the development work by Sanders and Sager, in which the successful application of the method to Cheddar cheese and to fluid milk was demonstrated, it became apparent that the enzyme remains active in all dairy products that have not been pasteurized, and therefore that the quantitative measurement of its activity could be applied to all dairy products, with, however, suitable adjustments of the reagents to compensate for the different buffering capacities of the various products. Accordingly, the Referee on Dairy Products recommended (5) that the Associate Referee study the development of a unified method applicable to various dairy products.

The following is a description of the laboratory method of Sanders and Sager for testing various dairy products to determine the adequacy of pasteurization. It includes those modifications needed to produce uniformly quantitative phosphatase values under fixed, standard conditions in applying the test to various common varieties and kinds of cheese and to fluid milk, cream, ice cream mix, sherbet mix, chocolate drink, butter, sweet buttermilk, cultured buttermilk, fermented milk drinks, goats' milk, and cheese whey. The modifications for different products are based on the results of extensive research in the laboratories of the Bureau of Dairy Industry, involving large numbers of tests on the various products, subjected to known heat treatments; also on collaboration and communications with the National Cheese Institute and many of its member firms and individuals, with Directors of Research in numerous dairy products firms, with public health laboratories in various States and cities, with research workers in several Agricultural Experiment Stations, and with Dr. F. W. Gilcreas and William Horwitz.

PHOSPHATASE TEST FOR PASTEURIZATION

REAGENTS

1. *Buffers:*

a. *Barium borate-hydroxide buffer.*—Dissolve 25.0 g of C. P. barium hydroxide [$Ba(OH)_2 \cdot 8H_2O$—fresh, not deteriorated] in distilled water and dilute to 500 ml. In another flask or cylinder dissolve 11.0 g of C. P. boric acid (H_3BO_3) and dilute to 500 ml. Warm each to 50°C, mix the two together, stir, cool to approximately 20°C, filter, and stopper the filtrate tightly (*p*H 10.6).

The buffer thus prepared is designated as the 25-11 buffer, the figures indicating the grams per liter of each of the respective reagents. Modifications in the quantities of these two reagents, necessary in preparing the appropriate buffers for testing various products, are indicated in Tables 1 and 2.

b. *Color development buffer.*—Dissolve 6.0 g of sodium metaborate ($NaBO_2$)[1] and 20 g of sodium chloride in water and dilute to 1 liter with water (*p*H 9.8).

c. *Color dilution buffer.*—Dilute 100 ml of color development buffer **1-b** to 1 liter with water.

d. *Standard borax buffer,* 0.01-molar, for checking *p*H meter, *p*H 9.18 at 25°C.[2] Dissolve 0.9603 g of pure borax (Bureau of Standards Sample 187) in distilled water (distilled recently or freshly boiled and cooled) and dilute to 250 ml. Keep stoppered tightly.

2. *Buffer substrates:*

a. *For evaluating pasteurization.*—Dissolve 0.10 g of phenol-free crystalline disodium phenyl phosphate[3] in 100 ml of the appropriate (Tables 1 and 2) barium borate-hydroxide buffer **1 a.**

b. *For quantitative results with raw milk and raw-milk products.*—Dissolve 0.20 g of the phenol-free crystalline disodium phenyl phosphate in 100 ml of the appropriate (Tables 1 and 2) barium borate-hydroxide buffer **1-a.**

3. *Protein precipitants:*

(a) *Zinc-copper precipitant for milk.* Dissolve 3.0 g of zinc sulfate ($ZnSO_4 \cdot 7H_2O$) and 0.6 g of copper sulfate ($CuSO_4 \cdot 5H_2O$) in water and dilute to 100 ml with water. The precipitant thus prepared is designated as the 3.0–0.6 precipitant.

b. *Zinc-copper precipitant for unripened cheese.* Dissolve 6.0 g of zinc sulfate and 0.1 g of copper sulfate in water and dilute to 100 ml with water. This precipitant is designated as the 6.0–0.1 precipitant.

c. *Zinc precipitant for ripened cheese and for butter.* Dissolve 6.0 g of zinc sulfate in water and dilute to 100 ml with water. This precipitant is designated as the 6.0 precipitant.

The quantities of the respective reagents to use in preparing the precipitants for

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[1] Obtainable from Amend Drug and Chemical Company, Inc., 117 East 24th Street, New York 10, N.Y.

[2] All *p*H values reported herein were determined at 25° C or corrected to that temperature.

[3] Obtainable, relatively pure, from Applied Research Institute, 2 East 23rd Street, New York 10, N.Y.

TABLE 1.—*Phosphatase test modifications for different kinds of cheese and cheese of different ages*

KIND OF CHEESE	AGE OR EXTENT OF CURING; OTHER DETAILS	BUFFER FOR OPT. *p*H (9.85–10.20)	PRECIPITANT	CRITERION, EXPERIMENTAL, PHENOL EQUIVALENT[a]
				mmg/0.25 g.
Cheddar, granular, stirred curd, hard cheese	<1 wk.	25-11[b]	6.0-0.1[c]	3
	1 wk.–1.5 mo.	25-11	6.0[d]	3
	1.5–4 mo.	26-11	6.0	3
	>4 mo.	27-11	6.0	3
Washed curd, soaked curd, Colby	<1 wk.	25-11	6.0-0.1	3
	1 wk.–2 mo.	25-11	6.0	3
	>2mo.	26-11	6.0	3
Swiss, Gruyère	<1 wk.	25-11	6.0-0.1	3
	1 wk.–1 mo.	25-11	6.0	3
	1–3 mo.	26-11	6.0	3
	>3 mo.	27-11	6.0	3
Brick, Muenster	<1 wk.	25-11	6.0-0.1	3
	1 wk.–1 mo.	25-11	6.0	3
	1–2 mo.	25-11	6.0	3
	>2 mo.	26-11	6.0	3
Edam, Gouda	<1 wk.	25-11	6.0-0.1	3
	1 wk.–2 mo.	25-11	6.0	3
	2–4 mo.	26-11	6.0	3
	>4 mo.	27-11	6.0	3
Blue mold, blue	<1 wk.	25-11	6.0-0.1	3
	1 wk.–1 mo.	26-11	6.0	3
	1–4.5 mo.	27-11	6.0	3
	>4.5 mo.	28-11	6.0	3
Camembert, Limburger	<1 wk.	25-11	6.0-0.1	4
	1 wk.–1 mo.	25-11	6.0	4
	1–2 mo.	26-11	6.0	4
	>2 mo.	27-11	6.0	4
Monterey	<1 wk.	25-11	6.0-0.1	3
	1 wk.–2 mo.	25-11	6.0	3
	>2 mo.	26-11	6.0	3
High moisture Jack	<1 wk.	25-11	6.0-0.1	3
	1 wk.–2.5 mo.	25-11	6.0	3
	>2.5 mo.	26-11	6.0	3

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[a] Values higher than those shown indicate under-pasteurization.
[b] Grams $Ba(OH)_2 \cdot 8H_2O$ and H_3BO_3, respectively, per liter.
[c] Grams $ZnSO_4 \cdot 7H_2O$ and $CuSO_4 \cdot 5H_2O$, respectively, per 100 ml.
[d] Grams $ZnSO_4 \cdot 7H_2O$ per 100 ml
[e] See also more sensitive modification in text (p. 316), alternative.
[f] Eight parts of 25-11 buffer plus 2 parts of water.

TABLE 1.—(*continued*)

KIND OF CHEESE	AGE OR EXTENT OF CURING; OTHER DETAILS	BUFFER FOR OPT. *p*H (9.85–10.20)	PRECIPITANT	CRITERION, EXPERIMENTAL, PHENOL EQUIVALENT[a]
				mmg/0.25 g.
Provolone, pasta filata	<1 wk.	25-11	6.0-0.1	3
	1 wk.–1 mo.	25-11	6.0	3
	1–3 mo.	26-11	6.0	3
	>3 mo.	27-11	6.0	3
Parmesan, reggiano, monte, modena, Romano, asiago old	<1 wk.	25-11	6.0-0.1	3
	1 wk.–2 mo.	26-11	6.0	3
	2–6 mo.	27-11	6.0	3
	6 mo.–1 yr.	28-11	6.0	3
	>1 yr.	29-11	6.0	3
Asiago fresh	Same as Cheddar			
Asiago medium	<1 wk.	25-11	6.0-0.1	3
	1 wk.–1 mo.	25-11	6.0	3
	1–3 mo.	26-11	6.0	3
	>3 mo.	27-11	6.0	3
Gorgonzola	Same as blue			
Cottage,[e] cook cheese, koch kaese	Dry	25-11	6.0-0.1	1
	Moist	25-11 (8+2)[f]	4.5-0.1	1
Cream cheese		25-11 (7+3)	4.5-0.1	3
Semi-soft cheese	<1 wk.	25-11	6.0-0.1	3
	1 wk.–1 mo.	25-11	6.0	3
	>1 mo.	26-11	6.0	3
Soft ripened cheese	<1 wk.	25-11	6.0-0.1	4
	1 wk.–1 mo.	25-11	6.0	4
	>1 mo.	26-11	6.0	4
Nokkelost, kuminost, sage cheese	<1 wk.	25-11	6.0-0.1	3
	1 wk.–1.5 mo.	25-11	6.0	3
	1.5–4 mo.	26-11	6.0	3
	>4 mo.	27-11	6.0	3
Pasteurized process, past. proc. pimiento, past. proc. with fruits, meats, etc.	Soft, mild	25-11	6.0	3
	Medium firm	26-11	6.0	3
	Firm, sharp (incl. Swiss, Gruyère)	27-11	6.0	3
Past. proc. cheese foods; past. proc.	Same as pasteurized			

TABLE 1.—(*continued*)

KIND OF CHEESE	AGE OR EXTENT OF CURING; OTHER DETAILS	BUFFER FOR OPT. pH (9.85–10.20)	PRECIPITANT	CRITERION, EXPERIMENTAL, PHENOL EQUIVALENT[a]
				mmg/0.25 g.
cheese foods with fruits, meats, etc.	process			
Past. proc. cheese spreads; past. proc. cheese spreads with fruits, meats, etc.	Soft, high moisture, incl. cream spreads	25-11	6.0	3
	Less soft, incl. blue	26-11	6.0	3
Cold pack, club; cold pack cheese foods; cold pack cheese foods with fruits, meats, etc.	Mild to medium flavored, soft	26-11	6.0	3
	Sharp, firm	27-11	6.0	3

testing other products, not mentioned under "Protein precipitants" above, are indicated in Tables 1 and 2.

4. *BQC* or *2,6-dibromoquinonechloroimine solution* (*Gibbs' reagent*).—Dissolve 40 mg of *BQC*[4] powder in 10 ml of absolute ethyl or methyl alcohol and transfer to a dark-colored dropper bottle. This reagent remains stable for at least a month if kept in the ice tray of a refrigerator. Do not use it after it begins to turn brown.

5. *Other reagents:*

a. *Copper sulfate, 0.05 per cent, for standards.*—Dissolve 0.05 g of copper sulfate in water and dilute to 100 ml.

b. *Butyl alcohol.*—Specify n-butyl alcohol, boiling point 116–118°C. To adjust the *p*H, mix 50 ml of the color development buffer **1-b** with a liter of the alcohol.

6. *Phenol standards:*

a. *Stock soln.*—Weigh accurately 1.0 g of pure phenol, transfer to a liter volumetric flask, dilute to a liter with water, and mix. One ml contains 1 mg of phenol. Use this stock soln to prepare standard solns. It is stable for several months in the refrigerator.

b. *Preparation of standards.*—Dilute 10.0 ml of the stock soln **6-a** to a liter with water, and mix. One ml contains 10 micrograms (0.00001 g; 10 mmg, or 10 units) of phenol. Use this standard soln to prepare more dilute standard solns; *e.g.*, dilute 5, 10, 30, and 50 ml to 100 ml with water to prepare standard solns containing 0.5, 1.0, 3.0, and 5.0 mmg or units of phenol per ml, respectively. Keep standard solns in the refrigerator.

In a similar manner, prepare from the stock soln as many more concentrated standard solns as may be needed, containing, for example, 20, 30, and 40 units per ml.

Measure appropriate quantities of the phenol standard solns into a series of tubes (preferably graduated at 5.0 and 10.0 ml) to provide a suitable range of standards as needed, containing 0 (control or blank), 0.5, 1.0, 3.0, 5.0, 10.0, etc., to 30 or 40 units.

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[4] Obtainable from Applied Research Institute.

To increase the brightness of the blue color and improve the stability of the standards, add 1.0 ml of 0.05% copper sulfate soln 5-a to each.

Add 5.0 ml of color dilution buffer 1-c and add water to bring the volume to 10.0 ml. Add 4 drops (0.08 ml) of BQC, mix, and allow to develop for 30 min. at room temperature. If the butyl alcohol extraction method is to be used in the test, extract the standards as described under "Conducting the test."

Read the color intensities with a photometer, subtract the value of the blank from the value of each phenol standard, and prepare a standard curve (straight line). When the standards are to be used for visual comparisons they should be stored in a refrigerator.

TABLE 2.—*Phosphatase test modifications for various dairy products other than cheese*

PRODUCT	QUANTITY OF SAMPLE	BUFFER FOR OPTIMAL pH (9.85–10.20)	PRECIPITANT	CRITERION, EXPERIMENTAL, PHENOL EQUIVALENT[a]
				mmg
Milk:				
Fresh	1 ml.	25-11[b] (5+5)[c]	3.0-0.6[d]	2/0.5 ml.
Old or slightly sour	1 ml.	25-11	6.0[e]	2/0.5 ml.
Cream:				
Fresh	1 ml. or 1 g.	25-11 (5+5)	3.0-0.6	2/0.5 ml. or 0.5 g.
Old or slightly sour	1 ml. or 1 g.	25-11 (8+2)	4.5	2/0.5 ml. or 0.5 g.
Ice cream mix	1 ml.	25-11 (8+2)	4.5-0.1	2/0.5 ml.
Sherbet mix	1 ml.	25-11 (5+5)	3.0-0.6	2/0.5 ml.
Chocolate drink	1 ml.	25-11 (8+2)	4.5-0.1	2/0.5 ml.
Butter	1 g.	18-8	6.0	2/0.5 g.
Sweet buttermilk	1 ml.	25-11 (5+5)	3.0-0.6	2/0.5 ml.
Cultured buttermilk and fermented drinks:				
Medium acid	1 ml.	25-11	6.0	2/0.5 ml.
Very acid, pH <4.5	1 ml.	26-11	6.0	2/0.5 ml.
Goats' milk[f]	3 ml.	27-11	7.5-0.1	1/1.5 ml.
Cheese whey	1 ml.	25-11 (5+5)	3.0-0.6	2/0.5 ml.

[a] Values higher than those shown indicate under-pasteurization.
[b] Grams $Ba(OH)_2 \cdot 8H_2O$ and H_3BO_3, respectively, per liter.
[c] Five parts of 25-11 buffer plus 5 parts of water.
[d] Grams $ZnSO_4 \cdot 7H_2O$ and $CuSO_4 \cdot 5H_2O$, respectively, per 100 ml.
[e] Grams $ZnSO_4 \cdot 7H_2O$ per 100 ml.
[f] Four-hour incubation period; use 7.0 ml of filtrate and add 3.0 ml. of color development buffer 1-b.

PHOTOMETRIC DETERMINATION

To read the color in aqueous soln, use a filter with maximum light transmission in the region of 610 mμ wave length.

To read the color in butyl alcohol, extract the color as described above and centrifuge the sample for 5 min. to break the emulsion and to remove the moisture suspended in the alcohol layer. A Babcock centrifuge can be adapted for this purpose by making special tube holders as follows: Slice a section ½ inch thick from a rubber stopper of suitable diameter to fit into the bottom of the centrifuge cup. Glue together two cork stoppers of appropriate diameter, bore through the center a hole of proper size to hold the tube snugly, and insert the double cork section into the cup. After centrifuging, remove nearly all of the butyl alcohol by means of a pipet with a rubber bulb on the top end. Filter the alcohol into the photometer cell and read with a filter with maximum light transmission in the region of 650 mμ wave length.

If more than approximately 4 ml of butyl alcohol is required for the photometer used, conduct the test in a larger tube and extract the color, in both the test and the standards, with the necessary quantity of butyl alcohol rather than with 5 ml specified above.

SAMPLING

1, Milk and other fluid products.—Mix the product well, pour several ml into a small tube, stopper the tube, and keep it in a refrigerator.

2. Hard cheese. Take a sample from the interior with a *clean* Roquefort trier, place in a small tube, stopper the tube, and keep it in a refrigerator.

3. Soft and semi-soft ripened cheese.—Harden the cheese by chilling it in the freezing chamber of a refrigerator. Taking special precautions to avoid contaminating the sample with phosphatase that may be present on the surface, use either of the following methods for sampling:

(a) Cut a portion from the end of the loaf or from the side of the cheese, extending in at least 2 inches if possible or to a point somewhat beyond the center in the case of a small cheese. Cut a slit ¼ to ½ inch deep at least halfway around the portion and midway between the top and bottom. Break the portion into two parts, pulling it apart so that it breaks on a line with the slit, being careful not to contaminate the freshly exposed, broken surface. Remove the sample from the freshly exposed surface at or near the center of the cheese.

(b) Remove the surface of the area to be sampled—*e.g.*, the end and the adjacent sides—with a clean knife or spatula, to a depth of ¼ inch. Clean the instrument and hands with hot water and phenol-free soap and wipe them dry. Remove the freshly exposed surface to a similar or greater depth, and repeat the cleaning. Then take the sample from the center of the freshly exposed area, preferably at or near the center of the cheese in the case of a small cheese.

4. Process cheese, spreads, butter, and other non-fluid products.—Take the sample from beneath the surface with a clean knife or spatula.

5. Ice cream and sherbet.—Melt the portion removed and allow it to remain melted for an hour or longer before testing, testing it as a fluid product.

Avoid the use of samples contaminated with mold.

6. Preservation.—If a preservative is necessary, for liquid products add 1 to 3 per cent of chloroform; for solid products put 1 to 3 ml of chloroform in the container, cover with a plug of cotton, insert sample and stopper container tightly. *Label preserved samples: "Poison, preservative added."*

CONDUCTING THE TEST

The chemical principles involved in the detection and measurement of milk phosphatase activity are the same for all dairy products. Some modifications, described below, have been found necessary for different dairy products, because of their differences in physical properties, compositions, and especially buffering capacities.

Cheese:

Step 1. Weigh, on a *clean* balance pan or watch glass, a 0.50-g sample (preferably in duplicate) and place in a culture tube 16 or 18×150 mm. Similarly, weigh another sample and place in a tube as a control or blank. If the cheese is sticky, weigh the sample on a piece of wax paper about 1×1 inch and insert the paper with the sample into the tube. Macerate the blank and the test with a glass rod about 8×180 mm.

Step 2. Add *to the blank* 1.0 ml of the appropriate (Table 1) barium buffer 1-a (without substrate added), macerate with the rod, leave the rod in the tube, heat for about a minute to at least 85°C (185°F) in a beaker of boiling water with the beaker covered so that the entire tube is heated to approximately 85°C, cool to room temperature, and macerate again with the rod.

Step 3. Add *to the test* 1.0 ml of barium buffer substrate **2-a** or **2-b** (Table 1) and macerate.

From this point, treat the blank and the test in a similar manner.

Add 9.0 ml of the appropriate barium buffer substrate **2-a** or **2-b** (total, 10.0 ml added), and mix. The rod may be left in the tube during incubation; if it is removed at this point, cut a piece of filter paper ca 1×1 inch, wrap and hold it tightly around the rod, rotate the rod while withdrawing it from within the tube so as to wipe the rod clean, insert the paper with the adhering fat into the tube, and stopper the tube.

Step 4. Incubate in a water bath at 37–38°C for 1 hour, mixing or shaking the contents occasionally.

Step 5. Place in a beaker of boiling water for nearly a minute, heating to approximately 85°C (use a thermometer in another tube containing the same volume of liquid), and cool to room temperature.

Step 6. Pipet in 1.0 ml of the zinc precipitant **3-c** for ripened cheese, or the zinc-copper precipitant **3-b** for unripened cheese, and mix thoroughly (*p*H of mixture, 9.0–9.1).

Step 7. Filter (5-cm funnel, 9-cm Whatman No. 42 or No. 2 paper recommended) and collect 5.0 ml of filtrate in a tube, preferably graduated at 5.0 and 10.0 ml.

Step 8. Add 5.0 ml of color development buffer **1-b** (*p*H of mixture, 9.3–9.4).

Step 9. Add 4 drops[5] of BQC, mix, and allow the color to develop for 30 min. at room temperature.

Step 10. Determine the intensity of blue color by either of two methods:

a. *With a photometer:* Read the color intensity of the blank and that of the test, subtract the reading of the blank from that of the test, and convert the result into phenol equivalents by reference to the standard curve described under "Phenol standards." The butyl alcohol extraction method ordinarily is unnecessary when using a photometer.

b. *With visual standards:* For quantitative results in borderline instances, *e.g.*, tests yielding 0.5 to 5 units of color, extract with butyl alcohol **5-b**. Add 5.0 ml of the alcohol and invert the tube slowly several times, centrifuge if necessary to increase the clearness of the alcohol layer, and compare the blue color with the colors of standards in the alcohol.

With samples yielding more than 5 units, compare the colors in aqueous tests with those of aqueous standards.

Step 11. *Dilution method for quantitative results:* In tests that are observed to be strongly positive during color development—*e.g.*, 20 units or more—in which 4 drops of BQC may be much less than sufficient to combine with all of the phenol, pipet an appropriate proportion of the contents into another tube, make up to 10.0 ml with color dilution buffer **1-c**, and add 2 drops more of BQC in the case of an unripened product or 4 drops in the case of a ripened product. With each test, dilute and treat the blank in the corresponding manner. Dilute each strongly positive test thus until the final color is within the range of the visual standards or photometer. Allow 30 min. for color development after the last addition of BQC, and make the reading at the end of the 30-min. period. To correct, multiply by 2 for a 5+5 dilution, by 10 for a 1+9 dilution, and by 50 for a 1+9 followed by a 2+8 dilution.

Alternatively, to reduce the amount of yellow off color, add 2 instead of 4 drops of BQC after each dilution, and allow the color to develop. Then test the completeness of color development by adding a third drop. Repeat the dilution procedure until the addition of an extra drop does not cause any further increase in the amount of blue color.

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[5] For merely detecting under-pasteurization in testing unripened cheese, 2 drops are sufficient, provided the visual standards likewise are prepared with 2 drops.

Step 12. *Calculation and evaluation of result:* When using 0.5 g of solid sample and adding a total of 11.0 ml of liquid, multiply the value of the reading by 1.1 to convert it to units of color or phenol equivalents per 0.25 g of cheese. The result, if desired, may be converted to phenol equivalents per 1 g by multiplying by 4.4. Evaluate the result by comparing it with the criteria of pasteurization in Table 1.

Milk and other fluid products:

Step 1. Pipet a 1.0-ml sample (preferably in duplicate) into a tube and pipet 1.0 ml into another tube as a control or blank. In testing cream, the sample may be weighed (1.0 g) if desired; in testing goats' milk, pipet in a 3.0-ml sample (Table 2).

Step 2. Heat the *blank* to the temperature indicated under "Cheese: Step 2," and cool to room temperature. From this point, treat the blank and the test in a similar manner.

Step 3. Add 10.0 ml of barium buffer substrate **2-a** or **2-b** (Table 2), stopper the tube, and mix.

Steps 4 to 11, inclusive. Follow the directions given for the corresponding steps under "Cheese" above, substituting the appropriate precipitant (Table 2) in Step 6, and, for merely detecting under-pasteurization, using 2 rather than 4 drops of BQC in Step 9.

Step 12. *Calculation and evaluation of result:* When using 1.0 ml of fluid sample and adding 11.0 ml of liquid (total liquid 12.0 ml, 5.0 ml of filtrate used), multiply the value of the reading by 1.2 to convert it to phenol equivalents per 0.5 ml of sample. If desired, the result may be converted to phenol equivalents per 1 ml by multiplying by 2.4. Evaluate the result by comparing it with the criteria of pasteurization in Table 2.

Butter:

Step 1. Weigh, on a piece of wax paper about 1 × 1 inch on a balance, a 1.0-g sample (preferably in duplicate) and insert the paper with the sample into the tube. Similarly, weigh another sample and place in a tube as a control or blank.

Step 2. Heat the *blank* to the temperature indicated under "Cheese: Step 2," and cool to room temperature. From this point, treat the blank and the test in a similar manner.

Step 3. Add 10.0 ml of barium buffer substrate **2-a** or **2-b** (prepared with 18-8 barium buffer, Table 2), stopper the tube, and mix.

Steps 4 to 11, inclusive. Follow the directions given for the corresponding steps under "Cheese" above, mixing the contents frequently and thoroughly during incubation, substituting the appropriate zinc precipitant (Table 2) in Step 6, and for merely detecting under-pasteurization, using 2 rather than 4 drops of BQC in Step 9.

Step 12. *Calculation and evaluation of result:* When using 1.0 g of butter and adding 11.0 ml of liquid, multiply the value of the reading by 1.1 to convert the result of phenol equivalents per 0.5 g of butter. Evaluate the result by comparing it with the criterion of pasteurization in Table 2.

MODIFICATIONS FOR DIFFERENT CHEESES AND OTHER DAIRY PRODUCTS

Different dairy products, and different kinds of cheese and cheeses of different ages, have different buffering capacities, and therefore some of them require modification of concentrations of the reagents. The modifications of the barium buffer needed to produce optimal *p*H conditions during incubation (9.85–10.20), and of the precipitant to yield uniformly clear filtrates and to minimize interference during color development under optimal *p*H conditions (9.3–9.4), are specified in Tables 1 and 2.

With some samples, especially cheese samples of unknown history, slight deviations from the optimal *p*H range may occur, but such deviations do not very materially affect the results. For example, *p*H values as low as 9.6 or as high as 10.35 during incubation have been found to result in an average decrease of not more than 20 per cent below the maximum in the quantity of phenol liberated. The use of the 25-11 buffer substrate with samples for which the 27-11 buffer substrate is specified yields *p*H values not lower than 9.8.

A trace of cloudiness in the filtrate, following the use of the precipitant as prescribed, indicates that the concentration of barium hydroxide in the buffer was not sufficiently great, *i.e.*, that the buffer substrate was not sufficiently alkaline. For example, the 25-11 buffer diluted 5+5 with water, for use with fresh milk, may yield a cloudy filtrate if used with old milk having a *p*H below approximately 6.0, or with milk that has soured, and the test should be repeated with a more concentrated buffer and precipitant. Likewise, the 25-11 buffer, for use with unripened cheese, may yield a cloudy filtrate if used with ripened cheese, indicating that the concentration of the buffer used was not sufficient. Increasing the concentration of zinc sulfate in the precipitant also eliminates turbidity of the filtrate.

In testing cheese of unknown history or age, information as to the percentage of solids, especially the nonfat solids, is useful as an indication of the correct buffer to use; cheese with a relatively high percentage of nonfat solids generally requires the use of a relatively concentrated buffer to adjust the *p*H of the mixture correctly.

For precise quantitative results on unknown samples, adjust the *p*H to 10.0–10.05 for the incubation.

Cottage cheese curd is heated in the presence of considerable acid during manufacture, and therefore its phosphatase values are comparatively low. Alternatively, to increase the sensitivity of the test on cottage cheese, apply the following modifications: Use a 1.0-g sample, 27-11 buffer substrate, 2-hour incubation, 6.0-0.1 precipitant, and a pasteurization criterion of 2 units per 0.5 g.

Phosphatase activity is much less in goats' milk than in cows' milk. The details of a modification designed to increase the sensitivity of the test applied to goats' milk are specified in Table 2.

To test concentrated milk products, reconstitute the product with water to its original concentration of milk solids and test in the manner specified for the original product.

To test for the presence of microbial phosphatase, which is indicated by blue color in the blank prepared as directed above, repeat the determination, adding 1 ml of the appropriate barium buffer (without substrate) to the blank and heat it for 5 min. in boiling water in a covered beaker. If the blank treated thus is negative, it indicates that the blue color in the original blank was due to microbial phosphatase, *i.e.*, a "false positive" sample.

PRECAUTIONS

The length of time that the crystalline disodium phenyl phosphate and the BQC powder will remain stable can be increased greatly by keeping them in the freezing chamber of a refrigerator.

The glassware, stoppers, and sampling tools should be scrupulously clean, and it is desirable to soak them in hot, running water after cleaning.

The bottles containing solid barium hydroxide and the barium buffer must be kept stoppered tightly to prevent absorption of carbon dioxide.

Phenolic contamination from plastic closures on reagent bottles has been encountered, and therefore the use of plastic closures should be avoided. Rubber stoppers should not be used in flasks in which butyl alcohol is stored. Glass or cork stoppers should be used.

DISCUSSION

In this work phosphatase activity caused by microorganisms has not been found in any fresh or reasonably fresh products. It has been encountered in some samples of old butter and old cream, on the surfaces of some soft and semi-soft ripened cheeses, and in several specific cultures of microorganisms. Such activity, in all samples tested to date, is indicated by blue color in the controls or blanks heated as described above. This seems to indicate, as others have pointed out earlier in tests on a few strains of microorganisms, that microbial phosphatases require a higher temperature for inactivation than milk phosphatase, and provides a means for tentatively differentiating between the two types of phosphatases.

The application of the method of Bessey *et al.* (4) for blood serum was investigated. It was found that the substrate, *p*-nitrophenyl phosphate, decomposes relatively rapidly under the influence of heat when the controls and tests are heated after incubation, whereas disodium phenyl phosphate undergoes relatively little hydrolysis when heated. Moreover, in tests prepared with known concentrations of raw milk in pasteurized milk, the photometric readings of the yellow color at 400 mμ with *p*-nitrophenyl phosphate were considerably smaller than those of the blue color at 610 mμ with disodium phenyl phosphate. The color produced with the latter substrate was much more distinct and the results much easier to interpret precisely. Whereas the enzymic activity in a concentration of 0.1 per cent of raw milk added to pasteurized milk could be detected visually by means of the Sanders-Sager method, and a concentration of 0.05 per cent could be detected with a photoelectric colorimeter, precise detection could not be made of quantities smaller than approximately 1 per cent by means of the method of Bessey *et al.*

The method of Bray and King (6), and also the modification described by Huggins and Talalay (7), involving the hydrolysis of phenolphthalein phosphate (colorless) yielding alkaline phenolphthalein (pink), were also investigated. However, even less precision was obtained with phenolphthalein phosphate as a substrate. The rate of hydrolysis of this substrate by the enzyme is too slow for a practical test for dairy products, and the pink color fades rather rapidly. For precise, quantitative determinations, both of these substrates were found less suitable than disodium phenyl phosphate.

All of the available time of the Associate Referee during the year has been devoted to research necessary for the development of the method and incidental cooperation with firms and individuals in the dairy industry desiring to use the method, and formal collaborative work has not been undertaken. Collaborative work on the use of the method for various dairy products should be done during the coming year.

RECOMMENDATIONS*

It is recommended—

(1) That, on the basis of the successful results reported on the test applied to Cheddar cheese and fluid dairy products by the Associate Referee, and the favorable results of collaborative work reported on the test applied to hard cheese by Gilcreas and applied to the soft cheeses by Horwitz, the Sanders-Sager phosphatase test be made an official method for testing fluid milk and cream, Cheddar type cheeses, and the soft unripened cheeses, as an index of the adequacy of pasteurization.

(2) That the test be made a tentative method for testing the various other dairy products for which a pasteurization test may be needed, including the other types of cheese, ice cream mix, sherbet mix, chocolate drink, butter, sweet buttermilk, cultured buttermilk, fermented milk drinks, goats' milk, cheese whey, and concentrated milk products; also that further collaborative work be done thereon during the coming year.

(3) That the present phosphatase test for pasterurization, **22.43–22.57**, inclusive, be dropped.

REFERENCES

(1) Sanders, G. P., and Sager, O. S., *J. Dairy Sci.*, **29**, 737 (1946).
(2) Gilcreas, F. W., *This Journal*, **30**, 422 (1947).
(3) Horwitz, Wm., *This Journal*, **30**, 430 (1947).
(4) Bessey, O. A., Lowry, O. H., and Brock, M. J., *J. Biol. Chem.*, **164**, 321 (1946).
(5) Frary, Guy G., *This Journal*, **30**, 418 (1947).
(6) Bray, J., and King, E. J., *J. Path. and Bact.*, **55**, 315 (1943).
(7) Huggins, C., and Talalay, P., *J. Biol. Chem.*, **159**, 399 (1945).

No reports were given on frozen desserts (gelatine and gums), frozen desserts (composition), chlorine in milk, or acidity of milk.

REPORT ON THE PREPARATION OF BUTTER SAMPLES

By H. J. Meuron (Food and Drug Administration,
San Francisco, California), *Associate Referee*

This report deals with a continuation of the investigation initiated by the predecessor Associate Referee.[1] He devised a plan for comparing and evaluating two rapid procedures for preparation of butter and oleomargarine samples as well as for testing a proposed rewording of the present official method. He subjected the plan of investigation to preliminary trial in the hands of collaborators in this laboratory. The results obtained indicated that this approach to the problem would yield information from which reasonable conclusions could probably be reached if more extended data were similarly obtained.

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* For report of Subcommittee C and action by the Association, see *This Journal*, **31**, 50 (1948).

[1] Vorhes, Frank A., *This Journal*, **29**, 119 (1946).

Accordingly the writer has solicited aid from as many sources as had expressed active interest. He has been fortunate in obtaining the cooperation of nine collaborators from eight different laboratories, which, incidentally, happen to be well distributed as to geographical location and size.

Instructions to collaborators were the same as those summarized in the previous report and in addition included the following:

Comments of collaborators are solicited for the purpose of obtaining a consensus of opinion as to:

(1) Whether the proposed rewording of the official method[2] represents as accurately as is practicable the actual technique employed (or that which should be employed) under present wording, except for specified temperature limits.

(2) Which of the rapid methods[3] (if either) is more convenient and rapid.

(3) Which of the rapid methods[3] (if either) is preferred for reasons other than convenience, rapidity, effectiveness and reliability; for example, in which procedure has the collaborator greater confidence solely as a result of using it.

The following constructive excerpts, from comments received, provide a basis for at least partial conclusion as to the consensus of opinion sought. The remarks of collaborators follow the order in which their results appear in Table 1, the form of which is the same as that employed in the tabulation presented in the previous report.

COMMENTS BY COLLABORATORS[4]

C. E. Hynds, Food Laboratory, N. Y. State Department of Agriculture & Markets, Albany, New York.

The proposed wording [of the official method] seems to accurately describe the technique used except the words "shake vigorously . . . until sample cools." It seems more likely that "shake vigorously at intervals . . . until sample cools" would be more appropriate. Method III seems more convenient and faster and the mixer is easier to clean. I prefer Method III.

H. S. Mitchell (VCM), Swift & Co., Union Stock Yards, Chicago, Ill.

Prefer Method III because of ease of mixing sample. Found that Methods I and II require from 15 to 30 minutes hand shaking for the preparation of the sample.

George E. Keppel, Minneapolis Station.

Method I represents accurately the technique used in following the present official method. I shake the jars intermittently until the contents cool to a creamy or "semi-solid" consistency. In addition, most of the analysts here give the butter an additional mixing for about 10 seconds after the jar is opened, using a long-handled spoon, and withdrawing portions for analysis with the same spoon.

I believe Method III is more convenient than Method II, in that the mixing blade is easier to clean between subs, and it is easier to incorporate butter adhering to the sides of the jar. Both methods are about equally rapid. Further, I believe Method III is more effective and reliable. I had never used either Method II or III before this, and noticed that in Method II, the paddles on our stirrer did not fit well in the pint Mason jars, being apparently intended for a taller jar. Therefore, I had to tilt the jar considerably to get at the butter under the jar shoulders, and the lower corners could not be reached. I am not certain that either Methods II or III offer much saving of time over the official method. In this laboratory as a general rule, we start analysis of butter almost as soon as it arrives. If the sample can be allowed to stand overnight, Method III has a distinct advantage. However, if analysis must be started at once, it requires almost as long to temper the butter evenly to 23°C. as it does to warm it to 35° (official method).

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[2] Method I, contained in previous report, *This Journal*, 29, 125 (1946).

[3] Method II—Present tentative method—*Methods of Analysis*, 1945, 333, sec. 22. 109-10. Method III—*This Journal*, 29, 125 (1946).

[4] With only 2 exceptions, collaborators were all in Food and Drug Administration.

Albert L. Weber, New York Station:

Method I is practically the same as that followed here. E-Z seal glass top Mason jars and International shaking machines have been used for some time and found to be satisfactory for mixing the samples. Shaking machines are essential when a large number of samples must be analyzed within 24 hours after collection. No material differences in results have been noted when comparing the shaking machine with hand shaking.

As to Methods II or III, neither shows much advantage in convenience or speed over the other. We feel that Method II may give better mixing. Both of these methods are too slow for our mass production needs. The time taken to soften the butter by Methods II and III is longer than that required by the official method, particularly if the butter is hard frozen initially [and shaking machines are used].

Meyer Matluck, Boston Station:

The third from the last sentence in Method I, "when optimum fluidity is attained . . . " is not clear. In actual practice, even though the sample has cooled to a "homogeneous semi-liquid of the consistency of thick cream," it may still flow down the side of the jar even though a layer does remain which obscures ready location of the surface of the sample. The deletion of the phrase " . . . no longer flows down the side of the jar" would, I believe, eliminate confusion without changing essential technique.

Assuming an average room temperature of 25°C., the duration of shaking required to go from optimum fluidity to a semi-liquid is in the neighborhood of an hour or more. On a hot summer day, room temperature of 28°, such as is frequent here, some artificial cooling, whether by shaking in a refrigerator or before an open window with an off-the-water breeze blowing in, is absolutely necessary. How can one reconcile such a situation with the admonition against employing artificial aids to cooling? The conditions described in Method I are such as to render it prohibitive from the standpoint of time consumption and physical exertion.

Methods II and III seem equally rapid and convenient. Method II, having a cover, minimizes moisture loss during preparation. Method III allows one to scrape all surfaces of the jar. Perhaps a cover could be adapted to this method. I feel that both Methods II and III are effective and reliable and far superior to the official method.

Herman O. Fallscheer, Seattle Station:

Method III is definitely more convenient and somewhat more rapid than Method II, and is also easier on the nerves, as Method II is quite noisy and "chattery." I would place more confidence in Method III than II. The blades in Method II don't always adequately mix the butter in the corners and shoulder of the jar, particularly if some separation has taken place in heating the sample.

John F. Armstrong, Los Angeles Station:

Method III is most desirable because it is rapid, smooth in operation (less chance of chipping pieces of glass into the sample), efficient (mixes the sample instead of boring a hole into it), and is physically easy as it eliminates laborious shaking.

Howard M. Bollinger, Los Angeles Station:

I found it impossible to get a visibly thorough mixing using Method II. After 5 minutes stirring darker yellow streaks were still observed on the interior surfaces of the jar, especially in the lower crevice where the butter was hardly disturbed regardless of the attempted thoroughness.

Our shaking machine can be adjusted so that the agitation is no greater than hand shaking and when the butter jars are placed on their sides a continuous washing action takes place without incorporation of air to make foam. I prefer Method III to Methods II and I, even when a shaking machine is used with the latter.

E. H. Zilliox, State Chemical Laboratory, Vermillion, South Dakota:

Only Methods I and III were used as the equipment for Method II was not available. Using Method I, it was necessary to shake the jars in the open doorway of a refrigerator as the room temperature was 90°F. Only enough toweling was folded around the jar to permit holding it. I do not favor Method I for it is too laborious especially when the room temperature is hovering around the century mark.

Method III is convenient and produces a very uniform sample. However, because of the chance of loss of moisture, I believe Method I should be used.

With minor changes, which are reflected in the accompanying recommendations, there is general agreement that the proposed rewording of the official method represents as accurately as is practicable the actual technique that should be employed.

Of the collaborators who commented on the comparative convenience of the two rapid methods, the majority (6) prefer Method III and the minority (2) conclude that the two methods are equally convenient, although one of the latter prefers the official method with the proviso that a shaking machine be employed. The collaborators whose results appeared in last years' report all prefer Method III.

From responses to the third question it was hoped to discover which, if either, of the rapid methods impart to the analyst the greater confidence. This would be a useful quality in a method for sample preparation in that, if supported by experimental results, it would provide the analyst some basis for deciding when the mixing had been completed. It was perhaps optimistic to expect that a definite and well grounded conclusion in this regard could be reached on initial application of a method. Nevertheless, the majority of collaborators give cogent reasons for their choice and, of these, four express greater confidence in Method III, while one comments favorably as to Method II. One collaborator cites a reason for some degree of lack of confidence in each of the rapid methods.

USE OF SHAKING MACHINES

Collaborator Weber's remarks on the use of shaking machines in the application of the official method, and the comments of others in regard to artificial aids to cooling, bear on certain general observations which were included within the instructions to collaborators, as follows:

> Our experience with this study to date indicates that the reworded official method has been found mechanically difficult. On two occasions collaborators have endeavored to employ a shaking machine to obviate the extended exertion of hand shaking. In one instance the results were quite comparable to those obtained by hand shaking, but in the other instance the results failed to check by as much as 2%. The latter experience is in agreement with our own. We observed that the motion of the "International" shaking machine, when vigorous enough to effect mixing, tended to produce a foamy texture which possibly accounts for the poor results obtained. Under the circumstances, we must view the use of a shaking machine with disfavor at this time.
>
> On one occasion a collaborator found it impossible to cool the sample while shaking at the temperature of the laboratory. He adopted the expedient of shaking it in the open doorway of a refrigerator, which proved successful. He subsequently expressed the opinion that the warmth of his hands on the jar may have prevented cooling. He suggested that a folded towel wrapped around the jar might have protected it against his body heat and permitted cooling at laboratory temperature. If other collaborators encounter such difficulty it is requested that the suggested expedient of wrapping the jar be tried. Please report such experience in full in order that consideration may be given to appropriate modification of the rewording of the method.

The writer has been unaware of any widespread use of shaking machines for this purpose. He and his associates had given the matter some consideration but the unfavorable initial results, cited above, discouraged immediate exploration of the potentialities of the modification. Moreover, our brief experience inclined us to the conclusion that in actual practice the use of shaking machines would be unduly cumbersome.

Collaborator Weber's remarks are entirely at variance with these conclusions. He speaks for a large laboratory that is accustomed to handling a heavy load of samples and states that the practice he describes has been in use by that laboratory over an extended period. Presumably the technique obviates the need for artificial aids to cooling.

This favorable experience, and the prospect of so revising the official method as to render it comparable to the rapid methods in convenience, would seem to warrant investigation with a view to developing supporting collaborative data. Pending successful outcome of such a study, however, the Associate Referee is not justified in modifying the wording of the official method in a manner to provide for mechanical shaking.

For the present this leaves the official method in the status of being not only inconvenient but, under certain climatic conditions, virtually impracticable. This situation need not necessarily be untenable, however, if either or both of the rapid methods qualify for official recognition by the Association.

COLLABORATIVE RESULTS

The nature of this investigation was such that collaborative samples could not be distributed by the Associate Referee. Each collaborator provided and prepared his own sample. Since each sample therefore represents entirely different butter, the determined per cent moisture, and, to a lesser degree, the variations and moisture losses cannot be compared in an absolute sense, but only relatively. Futhermore, each result is influenced by factors other than those of incomplete preparation and moisture loss. Therefore it is important to recognize that only the trend of the variations and moisture losses is strictly comparable.

Table 2 is a re-presentation of the variation figures from Table 1, combined with those of the previous report and arranged for convenient comparison.

The variation within each group of 6 results by Method II is less than that within either of the two corresponding groups of 6 results by Method I, in 1 out of the 17 sets.

The variation within each group of 6 results by Method III is less than that within either of the two corresponding groups of 6 results by Method I, in 8 out of the 18 sets.

The variation within each group of 6 results by Method III is less than that for the corresponding group of 6 results by Method II, in 10 out of the 17 sets.

Comparing the variations in another way:—the maximum variation is shown by Method I in 5 out of the 17 sets; by Method II in 7 out of the 17 sets; by Method III in 4 out of the 17 sets.

By both comparisons the trend appears slightly less favorable to Method II; by one comparison the indicated trend is slightly favorable to Method I and by the other comparison, slightly favorable to Method III.

These indications, however, are based on differences of very small magnitude. The mean variation for Method I is only .056% (standard deviation .031); for Method II the mean variation is .086% (standard deviation .037); and for Method III the mean variation is .060% (standard deviation .056). To conclude that one procedure is more effective than another, in producing a uniform mixture, on the basis of differences in results of the order of three or four hundreths of a per cent is to ascribe to the error of the moisture determination itself, an insignificance probably not in accord with the facts. At any rate it can be safely concluded that the variations fail to reveal any substantial lack of effectiveness, on the part of any one of the methods, to produce a practically uniform mixture.

Table 3 re-presents the moisture loss figures from Table 1 combined with those of the previous report and arranged for convenient comparison. Some of the figures are expressed in the negative sense, indicating a calculated moisture gain. This apparent anomaly must arise from the influence of extraneous factors, such as, for example, a difference in composition of the subdivisions or the normal analytical error of the moisture determination.

The moisture losses were calculated in both of the ways described in the previous report. The two types of calculation indicate a similar trend in the majority of the sets of four results, *i.e.*, where the first calculation indicates a higher or a lower moisture loss for one method, as compared to the

TABLE 1.—*1947 collaborative results in study of methods for preparation of butter samples*

COLLABORATORS			C.E.H.	H.S.M.	G.E.K.		A.L.W.	M.M.	H.O.F.	J.F.A.	H.M.B.	R.H.E.
			% Moisture determined on subdivisions initially prepared by indicated method									
Sub.	Meth.											
A	I	Max.	15.55	16.04	16.48	16.19	18.39	16.59	16.68	16.63	16.57	16.27
		Min.	15.44	15.97	16.45	16.16	18.28	16.50	16.64	16.00	16.52	16.24
		Ave.	15.507	16.010	16.458	16.182	18.327	16.540	16.655	16.610	16.537	16.252
		Var'n	.11	.07	.03	.03	.11	.09	.04	.03	.05	.03
B	I	Max.	15.07	16.07	16.51	16.17	18.41	16.58	16.68	16.66	16.60	16.27
		Min.	15.02	15.97	16.49	16.14	18.34	16.50	16.64	16.60	16.57	16.17
		Ave.	15.034[1]	16.022	16.502	16.152	18.357	16.522	16.657	16.634	16.590	16.207
		Var'n	.05	.10	.02	.03	.07	.08	.04	.06	.03	.10
Ave. Subs A & B			15.271	16.016	16.480	16.167	18.342	16.531	16.656	16.622	16.563	16.229
C	II	Max.	15.30	16.01	16.53	16.12	18.48	16.59	16.63	16.59	16.58	
		Min.	15.16	15.85	16.43	15.97	18.35	16.51	16.61	16.53	16.50	
		Ave.	15.227	15.962	16.493	16.032	18.412	16.550	16.623	16.562	16.551	
		Var'n	.14	.16	.10	.15	.13	.08	.02	.06	.08	
		Loss	.044	.054	−.013	.135	−.070	−.019	.033	.060	.012	
D	III	Max.	15.39	16.01	16.44	16.09	18.41	16.54	16.63	16.61	16.57	16.15
		Min.	15.36	15.84	16.38	16.07	18.17	16.48	16.60	16.55	16.53	16.11
		Ave.	15.380	15.942	16.417	16.082	18.327	16.512	16.617	16.577	16.542	16.130
		Var'n	.03	.17	.06	.02	.24[2]	.06	.03	.06	.04	.04
		Loss	−.109	.074	.063	.085	.015	.019	.039	.045	.021	.099
			% Moisture determined on subdivisions reprepared by indicated method									
A	II	Max.	15.49	15.90	16.49	16.19	18.33	16.55	16.67	16.57	16.53	
		Min.	15.38	15.79	16.45	16.17	18.28	16.48	16.63	16.55	16.48	
		Ave.	15.438	15.850	16.467	16.177	18.302	16.515	16.648	16.554	16.506	
		Var'n	.11	.11	.04	.02	.05	.07	.04	.02	.05	
		Loss	.069	.160	−.009	.005	.025	.025	.007	.056	.031	
B	III	Max.	15.09	16.03	16.50	16.13	18.29	16.52	16.66	16.63	16.56	16.10
		Min.	15.02	15.91	16.49	16.11	18.19	16.46	16.64	16.59	16.53	16.05
		Ave.	15.057	15.967	16.503	16.117	18.247	16.493	16.652	16.609	16.541	16.068
		Var'n	.07	.12	.01	.02	.10	.06	.02	.04	.03	.05
		Loss	−.023	.055	−.001	.035	.110	.029	.005	.025	.049	.139

[1] Average of 5 determinations.—All others average 6.

[2] One determination was very low, the other 5 were nearly alike. If the low determination is discarded the variation is .09.

TABLE 2.—*Variations*

	1947 COLLABORATORS										1946 COLLABORATORS							
	BUTTER										BUTTER				OLEOMARGARINE			
	C.E.H.	H.S.M.	G.E.K.		A.L W	M M.	H.O F.	J.F A.	H M.B.	E.H.S.	L.H.MCR.	L.A S.	M.G.Y.	H.W.G.	L.H.MCR.	L.A.S.	M.G.Y.	H.W.G.
METHOD I	.11	.07	.03	.03	.11	.09	.04	.03	.05	.03	.05+	.05	.08	.05	.03	.02	.06	.02
	.05+	.10	.02	.03	.07	.08	.04	.06	.03	.10	.15	.05	.04	.03	.04	.09	.05	.03
METHOD II	.14	.16	.10	.15	.13	.08	.02	.06	.08	—	.07	.05	.07	.09	.06	.05	.08	.07
METHOD III	.03	.17	.06	.02	.24*	.06	.03	.06	.04	.04	.03	.07	.01	.04	.01	.07	.02	.08

Method I includes figures for—Subs A and B.
Method II includes figures for—Sub C.
Method III includes figures for—Sub D.
All figures are from a variation of 6 determinations, except those followed by a + which are from a variation of 5 determinations.
* See Note 2, Table 1.

TABLE 3.—*Moisture losses*

	1947 COLLABORATORS										1946 COLLABORATORS							
	BUTTER										BUTTER				OLEOMARGARINE			
	C.E.H.	H.S.M.	G.E.K.		A.L.W.	M.M.	H.O.F.	J.F.A.	H.M.B.	E.H.S.	L.H.MCR.	L.A.S.	M.G.Y.	H.W.G.	L.H.MCR.	L.A.S.	M.G.Y.	H.W.G
								FIRST WAY[1]										
METHOD II	.044	.054	−.013	.135	−.070	−.019	.033	.060	.012	—	−.021	.051	.025	.147	.085	.216	.045	.062
METHOD III	−.109	.074	.063	.085	.015	.019	.039	.045	.021	.099	.034	−.021	−.024	.051	.028	.089	.037	.036
								SECOND WAY[2]										
METHOD II	.069	.160	−.009	.005	.025	.025	.007	.056	.031	—	.070	.020	.036	.038	.072	.110	.103	.045
METHOD III	−.023	.055	−.001	.035	.110	.029	.005	.025	.049	.139	.090	.000	.043	.043	.054	.035	.021	.011

[1] Average moisture by Method I on Subs A and B less moisture by Method II on Sub C, and less moisture by Method III on Sub D.
[2] Average of moisture by Method I less Method II on Sub A and average of moisture by Method I less Method III on Sub B.

other, the second calculation yields the same comparison, in 13 out of the 17 sets.

An equal or lower moisture loss is indicated for Method III, in comparison to Method II, in 20 of the 34 sets of comparisons.

The mean calculated moisture loss for Method III is .031% (standard deviation .040); that for Method II is .052% (standard deviation .055).

The trend appears favorable to Method III. The differences upon which the comparisons are based are again small, even somewhat less substantial than in the variation comparisons. The order of magnitude of the moisture losses for either method is slightly less than that of the variations. The Associate Referee believes that such order of magnitude would seldom if ever be of consequence to the considerations of the regulatory official, nor for that matter, of consequence to the interests of those whose product the regulatory official is called upon to consider. On those rare occasions when this factor need be taken into account its influence upon the matter under consideration can be gauged from the findings herein presented.

As to the rapid methods, the Associate Reeferee concludes (1) that substantial preference has been expressed in favor of Method III; (2) that, if any choice based on the experimental evidence is justified, it is in favor of Method III; (3) that the experimental evidence is of a character and extent warranting consideration of Method III for official status, and (4) that, in view of these facts, there is no compelling reason for retaining Method II.

Method III is published in detail in the preceding number of *This Journal*, 31, 91 (1948), under "Changes in Methods of Analysis."

REWORDING OF THE OFFICIAL METHOD

The following modification of the reworded official method is intended to give effect to collaborators' suggestions, with which the Associate Referee agrees.

Soften entire sample in a closed, wide-mouth fruit canning jar (fitted with a flat metal-disc cover secured by threaded ring) by warming gradually in water bath or running water maintained at about 39°C., shaking intermittently to reincorporate any separated fat and to observe fluidity of sample as softening progresses. Optimum fluidity is attained at that point where the emulsion is still substantially intact, but the mixture moves freely on shaking, washing all surfaces and flowing freely down the sides of the jar under gravity, revealing the surface of the body of the sample almost immediately, and permitting visual observation as to whether all portions of the sample have become liquefied sufficiently to promote their incorporation in the body of the sample under influence of shaking. At a higher temperature the emulsion usually tends to separate on short standing. Avoid overheating. When optimum fluidity is attained, shake vigorously at frequent intervals until the sample cools to an homogeneous semi-liquid of the consistency of thick cream and flows only sluggishly down the sides of the jar, a layer remaining which obscures ready location of the surface of the body of the sample by inspection of the exterior. Running water, water baths, and other liquid aids to cooling may not be used but a cool

draft of air is permissible, if climatic conditions require, provided shaking is continuous, not intermittent. Weigh portion for analysis promptly.

ACKNOWLEDGMENT

Acknowledgement is gratefully extended to the collaborators whose names are given in connection with their comment.

RECOMMENDATIONS*

It is recommended—

(1) That the present official method for preparation of butter samples be reworded as described in this report.

(2) That the method for preparation of butter samples identified in this report as Method III and described above be adopted as official (first action).

(3) That the present tentative method for preparation of butter samples be dropped.

(4) That collaborative investigation of the application of shaking machines in execution of the present Official Method for preparation of butter samples be initiated under an Associate Referee familiar with such application.

No reports were given on tests for reconstituted milk or sour serum test.

REPORT ON MICROANALYTICAL METHODS FOR EXTRANEOUS MATERIALS IN FOODS AND DRUGS

By KENTON L. HARRIS (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.), *Referee*

Reports of the Associate Referees for Drugs, Spices, and Miscellaneous Products; Fruit Products and Beverage Materials; Baked Products, Cereals, and Eggs; and Vegetable Products are presented herewith. Each change proposed is identified by the paragraph number in the 6th Edition if the *Methods of Analysis* to which it refers. It is recommended that these changes be accepted by the Association.

It is recommended that collaborative study be assigned and completed as rapidly as possible on the methods that warrant such additional attention. Such a program should be inaugurated during the coming year.

In the absence of a complete report by the Associate Referee for Dairy Products the Referee proposes the following changes in **42.11**:

Line 3 change "Transfer ca 100 vegetable fragments . . . " to "Transfer up to 100 vegetable fragments. . . . "

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* For report of Subcommittee C and action by the Association, see *This Journal*, 31, 50 (1948).

RECOMMENDATIONS*

It is recommended—

(1) That the method (sec. **42.32**), discussed in the report of the Associate Referee on extraneous materials in baked products, cereals, and eggs, be adopted as official.

(2) That methods for extraneous matters in fruits be subjected to collaborative studies.

(3) That methods for extraneous matters in drugs be further studied.

(4) That the changes in wording of section **42.57** (mold in tomato products) providing for adjusting the total solids in the sample by the use of the Abbé refractometer, as recommended by the Associate Referee, be made.

(5) That the changes in wording of section **42.11** (manure fragments in dairy products), recommended by the Associate Referee, be made.

REPORT ON EXTRANEOUS MATERIALS IN DRUGS, SPICES, AND MISCELLANEOUS PRODUCTS

By WILLIAM V. EISENBERG (Food and Drug Administration,
Federal Security Agency, Washington, D. C.),
Associate Referee

It is recommended that all of the methods for ground spices, **42.78** through **42.84** inclusive, be reviewed in order to change the size of sample examined from 10 grams to 25 grams. The larger sample will be more representative for the detection of certain types of filth such as rodent hairs and excreta, which are often not uniformly distributed.

Our experience has shown that no physical difficulties will be encountered using the present methods for extracting 25 grams of black pepper, nutmeg, or ginger for microscopic counting of the filter papers. Cinnamon, because of its woody texture and extremely fine grind, introduces some difficulty due to the tissues floating and thus hampering the examination of the filter papers. Experimental work should be carried on next year to test the applicability of the other spice methods to change in size of sample from 10 to 25 grams.

Those methods for whole or cracked spices which presently use a 25 gram sample should also be reviewed for the purpose of changing the size of sample from 25 to 100 grams, for reasons similar to that stated above.

The spice methods and most of the microanalytical methods attempt to extract the so-called "light filth" consisting principally of insect and rodent filth simultaneously by means of one procedure. Recent experience has indicated that the defatting operation with petroleum benzine, which is often helpful in increasing the recovery of insect parts, will on the other hand decrease the recovery of rodent hairs. Duplicate analyses using methods **42.78** and **42.83** for whole black pepper seem to verify this premise. Further work, however, should be carried out which will include the examination of collaborative samples. Similarly, collaborative work should include other spices and methods with a view to evaluating the wisdom of using one and the same extraction for recovery of insect and rodent filth.

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* For report of Subcommittee C and action by the Association, see *This Journal*, 31, 51 (1948).

It is recommended—*

That methods for extraneous materials in drugs be further studied.

No report was given of extraneous materials in dairy products or nut products and confectionery.

REPORT ON EXTRANEOUS MATERIALS IN FRUIT PRODUCTS AND BEVERAGE MATERIALS

By F. Allen Hodges (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

Comparative studies were conducted using two methods for determining insect infestation in whole dried figs and two methods for determining insect contamination in fig paste. The methods used were (1) Macroscopic examination of 100 figs, (2) A gasoline flotation method on whole figs, (3) Fig paste prepared from 100 figs by putting the figs through a food chopper and using method **45.52** (A.O.A.C. 6th Ed. 1945), and (4) A dilution procedure of fig paste.

A. INFESTATION IN WHOLE DRIED FIGS

(1) The macroscopic method consisted of the examination of 100 figs (ca 1500 grams) by cutting open each fig and examining the interior for the presence of larvae and/or adult insects.

Table 1.—*Results by the macroscopic method*
(100 figs examined)

SAMPLE	A	B	C	D	E	F	G	H	AVE./100 FIGS
Larvae—2 mm and over	7	2	4	1	1	3	1	2	2.6
Larvae—less than 2 mm	6	3	3	3	1	1	0	3	2.5
Whole Insects	3	0	1	2	0	0	1	0	0.9
Larval Fragments	0	0	0	0	0	0	0	0	0.0
Larval Heads	0	0	0	0	2	1	0	0	0.4
Insect Fragments	0	0	0	0	0	0	0	0	0.0

(2) The gasoline flotation method used consisted of taking 100 figs and cutting them into halves. These halves were covered with water, heated, boiled for 10–15 minutes, and cooled to ca 50°. Ca 50–75 ml of

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* For report of Subcommittee C and action by the Association, see *This Journal*, 31, 51 (1948).

gasoline was added to the container and thoroughly stirred. Water was added so that the gasoline layer came to about 1″ from the top of the container. The container was allowed to stand 20–30 minutes with occasional stirring, and the larvae and insects which rose to the gasoline layer were picked out and examined under a wide field binocular microscope and counted.

TABLE 2.—*Results by the gasoline flotation method*
(100 figs examined)

SAMPLE	A	B	C	D	E	F	G	H	AVE/100 FIGS
Larvae—2 mm and over	10	10	15	4	5	5	12	10	8.9
Larvae—less than 2 mm	20	5	8	25	19	11	20	9	14.6
Whole Insects	6	2	5	1	1	0	4	1	2.5
Larval Fragments	15	0	2	1	3	7	4	1	4.1
Larval Heads	4	1	1	0	3	0	9	5	2.9
Insect Fragments	3	2	1	0	1	0	5	0	1.5

A comparison of method (1) and method (2) shows that gasoline flotation gave a higher recovery than did the manual macroscopic method.

B. INSECT CONTAMINATION OF FIG PASTE

(3) Method **42.52.** After passing the whole figs through a food chopper eight 100 g. samples were examined by this method and results are shown in Table 3. Since the figs were ground up into a paste many of the insects and/or larvae present will be broken up into fragments rather than remain whole. For this reason a large proportion of insect heads and fragments were found.

TABLE 3.—*Examination of fig samples method 42.52*
Fig paste

SAMPLE*	A	B	C	D	E	F	G	H	AVE./100 G.
Heads	9	9	12	3	8	5	7	15	3.6
Larvae—2 mm or over	0	0	1	0	0	1	1	0	0.2
Larvae—less than 2 mm	1	2	3	0	10	1	7	1	1.3
Miscellaneous insects	0	0	1	1	1	0	0	0	0.2
Larval fragments and larval skin fragments	17	6	11	0	12	5	5	32	4.6
Miscellaneous insect fragments	19	8	12	2	1	3	3	18	3.5

* Samples A–G each represented 2–100 g. portions; sample H was of 5–100 g. portions.

(4) In the dilution procedure fig paste was prepared from 100 figs and mixed with water in the proportion 1 part paste to 3 of water. To this

mixture was added 4% formalin. The examination consisted in using 400 g. portions of the diluted material. The procedure used in (3) above was followed except that the NaOH and HCl steps were omitted. Five samples of figs were examined using 400 g. portions. Results obtained are shown in Table 4. As in the case of method (3) above, a small number of whole larvae and/or insects were recovered since the figs were ground up into a paste.

TABLE 4.—*Examination of diluted fig paste*
(400 g. portions of 1:3 diluted material examined)

SAMPLE NO.	A	B	C	D	E	AVE./100 G. ORIGINAL PASTE
Larvae—less than 2 mm	1	1	0	0	2	0.8
Larvae—2 mm or over	3	2	0	1	1	1.4
Heads	13	11	4	3	0	6.2
Miscellaneous Insects	0	0	2	0	0	0.4

A comparison of method (3) (**45.52**) and method (4) shows that the NaOH and HCl treatment in **42.52** gave a more complete separation of insects than did the simple dilution of method "(4)."

DISCUSSION

The use of the gasoline flotation method in comparison with the other methods used showed much better recovery of insects, particularly whole larvae. It also eliminates the grinding up of the figs into a paste and for this reason whole larvae and insects are detected rather than insect heads and fragments. It requires less time to examine a sample and is easier to pick out the insects.

It is recommended* that collaborative work be done on the following method.

Examination of Whole Dried Figs—(Flotation)

INSECTS

Pick out 100 figs from each subdivision of sample to be examined. Cut the figs into halves and transfer to a large container. Cover with water. Heat. Boil for 10–15 min. or until the figs are soft. Allow to cool to ca 50°. Add ca 50 ml gasoline. Allow to stand ca 20–30 min., stirring occasionally during this period. Fill the container with additional water to within ca 1 inch from top.

Examine the surface of the beakers or container under a strong light using a magnifier of ca 5×. Pick out larvae, insects, and/or insect fragments which rise into the gasoline layer. Examine the insects found, using a Greenough type microscope at a magnification of ca 20×.

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* For report of Subcommittee C and action by the Association, see *This Journal*, 31, 51 (1948).

REPORT ON EXTRANEOUS MATERIALS IN VEGETABLE PRODUCTS

By FRANK R. SMITH (Food and Drug Administration, Federal Security Agency, Washington, 25 D. C.), *Associate Referee*

In the methods for extraneous materials in vegetable products only one slight revision seems necessary this year. In method **42.57** it is necessary to determine the total solids by an impractical and lengthy weighing and drying procedure. The method now reads in part, "in case of puree and paste mix H_2O to make total tomato solids of diluted product 8.37–9.37%." To obviate this necessity provision can be made to control the solids by refractometer.

This involves no change in the method, but such revision provides a practical way of securing these dilutions. It is recommended* that it be adopted for official, final action. The details of the proposed revision as it applies to the official method for molds in tomato products, section **42.57**, are given in *This Journal,* **31,** 118, 1948.

REPORT ON EXTRANEOUS MATERIALS IN BAKED PRODUCTS, CEREALS, AND EGGS

By KENTON L. HARRIS (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.), *Associate Referee*

Section **42.32** concerns the separation of rodent excreta pellet fragments from whole corn meal. The precursor of this method first appeared in a U. S. Food and Drug Administration mimeograph by Helsel and Harris (1939), was published in *Cereal Chemistry,* **18,** 655 (1941), and in its present form first appeared in the *Methods of Analysis,* Ed. 6, p. 781.

From time to time collaborative samples have been prepared and sent to various analysts. The results on these samples are given in Table 1 opposite. It should be noted that there are omitted from the table several counts reported by analysts inexperienced in the microscopic identification of the extracted pellet fragments. Samples consisted of 50 gram portions of meal taken from a well-mixed batch of grossly contaminated commercial corn meal.

The results of the collaborative work show a marked agreement between the various collaborators. The method is recommended* for adoption as official.

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* For report of Subcommittee C and action of the Association, see *This Journal,* **31,** 51 (1948).

TABLE 1.—*Results from various analysts on 50 gram samples of whole corn meal examined for rodent excreta pellet fragments*

SAMPLE NUMBER	ANALYST	RODENT PELLET FRAGMENTS COUNT
M49057	KLH (4 counts)	21, 27, 27, 25
	He	24
	Wo	23
	Co	20
	ME	27
	Lo	21
M49058	KLH (2 counts)	17, 18
	Wo	15
	ME	13
	QCR	18
	QSJ	12
	Lo	20
	Bo	18
	Coh	16
	Cou	16
	Ca	17
	Pi	15
M49059	KLH (4 counts)	25, 26, 33, 21
	Sa	21
	Yo	23
	QCR	20
	QSJ	24
	Lo	39
	Bo	33
	Coh	20
	Cou	19
	Ca	17

REPORT ON FISH AND OTHER MARINE PRODUCTS

By ANDREW M. ALLISON (Food and Drug Administration, Federal Security Agency, Boston 10, Mass.), *Referee*

In accordance with recommendations of Subcommittee C (*This Journal*, **30**, 51, 1947) collaborative studies have been continued on the Modified Roese-Gottlieb method for determination of ether extract in fish. The method has been modified considerably and a report by Associate Referee Voth is being submitted. It is recommended—*

(1) That the Modified Roese-Gottlieb, or acid extract, method for fat in fish be adopted as tentative.

(2) That the work on methods of determining total solids in fish be continued (*This Journal*, **26**, 226, 1943).

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* For report of Subcommittee C and action by the Association, see *This Journal*, **31**, 52 (1948).

REPORT ON ETHER EXTRACT IN FISH

By MENNO D. VOTH (Food and Drug Administration, Federal Security Agency, Boston, Mass.), *Associate Referee*

A digestion or acid extract method for the determination of fat in fish has been previously described (1).

Considerable additional work has since been done on this method. It has been improved and simplified. Specifically, the following changes have been made: 1. The charge has been increased from 5 grams to 8 grams. This 60 per cent increase helps to offset errors due to unequal fat distribution in the sample or to other causes. 2. The use of equal parts of ethyl ether and petroleum benzine has been substituted for the use of ethyl ether alone. This change eliminates the necessity of washing the ether extract and, therefore, simplifies the method. 3. The steam bath has been substituted for the hotplate or burner as a means of heating during digestion. This change eliminates all bumping, which constituted a real hazard, and also eliminates the need for specially treated sand.

The proper proportions of fish, acid, and alcohol used in the method were determined after numerous trials and are believed to be in the best possible ratio. The major difficulty encountered in the work was the residue not soluble in petroleum benzine which remained in the fat flask after drying, and which was found principally in low fat fish, such as haddock, cod, etc.

The original plan was to obtain the weight of the extracted fat by difference as is done in the Roese-Gottlieb method (2). However, it was found that fish fats extracted from low fat fish (such as haddock) by the usual fat solvents have a tendency to polymerize or solidify during heating in such a manner as to make them insoluble in petroleum benzine (or in any of the common fat solvents). This polymerization is believed to be affected by various factors. Some of these are: 1. Age of fish in storage. 2. Length of heating period after evaporation of the solvents. 3. Length of digestion period. 4. Lack of clarification of the extract (by centrifuging). Under certain conditions, this polymerization can be controlled. By taking special precautions (see Table 1), such as heating the fats for a minimum length of time in a vacuum oven or in an atmosphere of carbon dioxide, insignificant polymerized residues are obtained. It was concluded, however, that to include such precautions would make the method cumbersome. Any appreciable residue due to polymerization, which is obtained after washing with petroleum benzine, must be considered a fat, since experimental evidence shows that when certain precautions are taken, the residue obtained is insignificant. It is also to be noted that the weight of fat plus the residue remains constant within the limits of experimental error, regardless of the factors which increase or decrease the weights of those components. Some of the experimental data is shown in Table 1 below.

TABLE 1.—*Polymerized residues obtained from fat extracted from haddock flesh using the Roese-Gottlieb method*

EXTRACTED AND HEATED USING SPECIAL PRECAUTIONS			EXTRACTED AND HEATED WITHOUT USING SPECIAL PRECAUTIONS		
PER CENT FAT AND RESIDUE	PER CENT RESIDUE	PER CENT FAT BY DIFFERENCE	PER CENT FAT AND RESIDUE	PER CENT RESIDUE	PER CENT FAT BY DIFFERENCE
0.53	0.01	0.52	0.54	0.37	0.17
0.53	0.02	0.51	0.53	0.37	0.16
0.56	0.02	0.54	0.56	0.33	0.23
0.53	0.01	0.52	0.55	0.32	0.23
0.53	0.00	0.53	0.52	0.33	0.19
0.52	0.01	0.51	0.53	0.31	0.22
0.54	0.01	0.53	0.54	0.12	0.42
			0.53	0.40	0.13
			0.56	0.13	0.43
			0.53	0.27	0.26

It was also determined that 8 grams is the maximum possible charge that can be handled in the small Mojonnier tubes which are readily available. Considerable experimental work was done before this conclusion was reached. This was particularly true in low fat fish, such as haddock (see Table 2 below).

TABLE 2.—*Per cent of fat extracted from different weights of haddock by digestion*
(8 ml HCl used in each case)

Sub	1	2	3	4	5	6	7	8
Wt. of fish used (gms)	5	6	7	8	8	9	9	10
Per cent fat	0.57	0.57	0.57	0.58	0.58	0.53	0.52	0.48

For comparison, fat determinations by two other methods were made by the Referee on fish samples identical with those sent to collaborators. The first method used was that previously described (1). Ethyl ether alone is here used as the solvent. Results shown in the table below are comparable with those obtained by the revised method shown in Table 5.

Determinations were also made using the Stansby-Lemon continuous extraction method (3). As was expected, the results are a little higher than those obtained by the digestion method (see Table 3 below). In the Stansby-Lemon method, a crude extract is first obtained using acetone as the solvent. This extract is then dried and re-extracted with anhydrous ethyl ether. The slightest bit of moisture in the crude extract, solvent, or apparatus will dissolve some of the large quantity of water-soluble residue left behind during the re-extraction with the anhydrous ethyl ether. Even after taking the utmost precautions, such as a 5-hour vacuum oven drying period, a small portion of water-soluble material comes over as indicated by the brown-colored ether solution and the final dark viscous fat.

TABLE 3.—*Results on collaborative samples by two methods*
(Per cent fat)

	FRESH HADDOCK	Av.	FRESH MACKEREL	Av.	CANNED MACKEREL	Av.
Ethyl ether extraction method (1)	0.53 0.55	0.54	3.83 3.89	3.86	11.92 12.03	11.98
Acetone extraction method (3)	0.83 0.89	0.86	4.27 4.27	4.27	12.52 12.61	12.57

A limited study was made on the effect of a longer digestion period. Under ideal conditions, the fish are adequately digested in 60 minutes or even less on the steam bath. In order to determine whether a longer period would have a tendency to reduce the yield, two types of fish were digested on the steam bath for varying periods of time. It was found that within limits longer heating periods were not detrimental (see Table 4 below).

TABLE 4.—*Effect of digestion period on the steam bath on per cent of extracted fat*

HADDOCK			CANNED MACKEREL		
SUB	DIGESTION TIME IN HOURS	PER CENT FAT	SUB	DIGESTION TIME IN HOURS	PER CENT FAT
1	1	0.52	1	1	12.10
2	1.5	0.51	2	1.5	12.02
3	2	0.52	3	2	11.97
4	3	0.53	4	4.5	12.04
5	4	0.52			

Three samples of fish consisting of fresh haddock, fresh mackerel, and canned makerel were submitted for collaborative studies. The samples of fish which were sent to the collaborators for determination of fat were prepared in the same manner as described in the Referee's previous report (1). The revised method is as follows:

CRUDE FAT—ETHER EXTRACT

PREPARATION OF SAMPLE

Prepare the sample, according to the type of pack, as directed under par. **24.2** (*Methods of Analysis, A.O.A.C.*, 1945, 359) and keep ground material in a sealed jar. If the jar has been chilled, allow the sample to come to room temperature and shake jar so that any separated liquid will be absorbed by the fish. Open jar and stir contents with spatula, thoroly contacting the sides and lid so as to incorporate any separated liquid or fat.

METHOD

Weigh into a 50 ml beaker 8 grams of the well mixed sample. Add 2 ml of HCl. Break up coagulated lumps with a stirring rod having an extra large flattened end and continue until a homogeneous mixture is obtained. Add an additional 6 ml of HCl, mix, cover with watch-glass and heat on the steam bath for 90 min. with an occasional stirring. Cool solution and transfer to Mojonnier fat extraction tube. Rinse beaker with 7 ml of ethanol, add to extraction tube and mix. Rinse beaker with 25 ml of ethyl ether in three portions and add to extraction tube, stopper with cork or stopper of synthetic rubber unaffected by usual fat solvents, and shake tube vigorously for one min. Add 25 ml of petroleum benzine (b.p. below 60°) to extraction tube and repeat vigorous shaking. Centrifuge Mojonnier flask 20 min. at ca 600 r.p.m. and proceed as directed under par. **20.16** (*Methods of Analysis, A.O.A.C.*, 1945, 240) beginning "Draw off as much as possible of ether-fat soln."

Drying to constant weight takes ca 40 min. for fish. Long heating periods tend to increase the weight of the fat. If a centrifuge is not available, the extraction can generally be made by letting Mojonnier flasks stand until the upper liquid is practically clear, then swirling flask and again letting stand until clear. If a troublesome emulsion occurs, draw off as much of the ether-fat soln as possible after letting flask stand, add a ml or two of ethanol, swirl, and again allow mixture to separate.

DISCUSSION

The results obtained by the collaborators are given in Table 5. Good results were obtained.

TABLE 5.—*Collaborative results on fat*
(Per cent)

COLLABORATOR	DIGESTION METHOD					
	FRESH HADDOCK		FRESH MACKEREL		CANNED MACKEREL	
		Ave.		*Ave.*		*Ave.*
G. Kirsten	0.56		3.84		11.99	
	0.58	0.57	3.88	3.86	12.02	12.01
H. I. Macomber	0.55		4.03		11.92	
	0.56	0.56	4.04	4.04	11.67	11.80
S. H. Perlmutter	0.54		3.71		11.78	
	0.56	0.55	3.80	3.76	11.82	11.79
T. J. Klayder	0.56		3.62		11.95	
	0.53	0.55	3.65	3.64	11.90	11.93
F. C. Minsker	0.56		3.68		11.98	
	0.58		3.76		11.98	
	0.56	0.57	3.71	3.71	11.81	11.92
M. D. Voth	0.56		4.01		12.09	
	0.56	0.56	4.03	4.02	12.14	12.12
Mean		0.56		3.83		11.93
Ave. Deviation from Mean		0.01		0.13		0.10
Maximum Deviation from Mean		0.03		0.21		0.26

No unusual difficulties were experienced by any of the collaborators.

The Associate Referee wishes to thank the collaborators for their cooperation and help.

REFERENCES

(1) *This Journal*, **29**, 46 (1946).
(2) *Methods of Analysis, A.O.A.C.*, 1945, sec. **22.25**.
(3) *This Journal*, **27**, 237 (1944).

No reports were given on gums in foods.

REPORT ON SPICES AND CONDIMENTS

By SAMUEL ALFEND (Food and Drug Administration, Federal Security Agency, St. Louis, Mo.), *Referee*

Reports were submitted ty the Associate Referees on Vinegar, Mustard, and Mayonnaise and Salad Dressings.

VINEGAR

Associate Referee Loughrey has reworded the method for permanganate oxidation number for convenience and clarity, and has subjected it to collaborative study. The results were promising. The Associate Referee recommends another collaborative test before offering it for adoption as official, and the Referee agrees. The Referee has noted the precipitation of iodine, mentioned by Garfield,[1] and the release of iodine vapor above the solution, and suggests increasing the concentration of potassium iodide to dissolve all the iodine.

No work was done on mineral acids. The qualitative method for free mineral acids proposed by Rokita and Henry is excellent, but the quantitative method proposed last year[2] has obvious theoretical deficiencies. It was proposed to run total phosphates, sulfates, chlorides or nitrates in the sample, and correct for naturally occurring salts by subtracting the amount of the pertinent radicle found in the ash. It is obvious that some of the chloride would be lost as potassium chloride on ignition, and that potassium nitrate would break down on ashing, leaving little or none in the ash. It is also apparent that in the presence of free sulfuric or phosphoric acid, the potassium carbonate which constitutes much of the ash of apple or wine vinegar would be converted to the sulfate or phosphate, and the correction for the salts would be far too large. It might prove practicable, once the mineral acid adulterant has been identified, to determine the radicle in the vinegar, and apply a salt correction based on the normal content of the particular salt in that type of vinegar. The correction would be very small for distilled vinegar, and even for cider vinegar the possible error in the correction might not be important. Another approach might be the setting up of curves or nomographs relating *p*H, titratable acidity, and proportions of acetic and mineral acids.

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[1] *This Journal*, 30, 446 (1947).
[2] *This Journal*, 29, 304 (1946).

The Associate Referee has done some preliminary work on tartaric acid. This value is of interest primarily as a means of detecting adulteration of wine vinegar. Since the ability to detect dilution with other vinegars is limited by the variation in the natural content of tartrates in wine vinegar, it is worth while to seek methods depending on the presence of a characteristic constituent of the adulterant. Ey[3] has suggested the determination of sorbitol, a characteristic component of cider, as a means of detecting the presence of cider in grape wine. This should prove equally useful in detecting cider vinegar in grape vinegar, and the possibilities should be investigated.

Loughrey has pointed out the possibility of false positive tests for caramel in old cider vinegars, by the Lichthardt test, and has recommended study of other tests now being studied in wines. He expresses doubt as to the desirability of studying the Mallory and Love quantitative method before exploring the possibilities of the other tests. The Referee agrees.

MAYONNAISE AND SALAD DRESSINGS

Associate Referee Fine has submitted the results of collaborative work demonstrating that there is no detectable loss of starch when mayonnaise is held for a month at room temperature. The Referee endorses his recommendation that the method be made official (first action), and that no further work be done on it.

Final adoption of several methods has been held up pending final action on the adoption of the method for preparation of salad dressing samples as official. It was decided that it would be well to wait for several years, to determine if experience with the methods developed any unknown "kinks." Several field stations of the Food and Drug Administration have reported difficulty in removing the fat from mayonnaise prior to acid digestion in the determination of nitrogen, and Ferris and Macomber[4] have found that this difficulty can be overcome by adding 100 ml chloroform to the sample and evaporating off the chloroform, before extracting the fat with petroleum ether. Macomber has also reported difficulty in the determination of fat in salad dressings having less than 50 per cent of fat. He pointed out that the A.O.A.C. collaborative work has been done on samples containing more than 50 per cent of fat. It is believed that these points should be cleared up, and that the methods for preparation of sample, fat, and sugars should then finally be made official.

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[3] Detection of Cider in Grape Wine. Dr. Ey, *Schweiz. Apoth. Ztg.*, **83**, 605–607 (1945). Abstracted in *J.A.Ph.A.*, **36**, Abs., p. 12 (Jan., 1947).

[4] Unpublished reports.

MUSTARD

Associate Referee Garfield has submitted collaborative results on starch in mustard flour and prepared mustard which fully justify his recommendation that the method be adopted as official and no further work be done. Garfield has also done some preliminary laboratory work on the methods for the pungent principles in mustard (volatile oil, etc.), but is not yet ready to report on this subject.

Since the methods for starch in mustard and in salad dressing are essentially the same, after the preliminary treatment of the sample, it is suggested that wording agreeable to both Associate Referees be adopted for one method applicable to both types of food.

No report on volatile oil in spices was submitted by the Associate Referee.

RECOMMENDATIONS*

It is recommended—

(1) That studies on the application to vinegar of the Mathers and the A.O.A.C. confirmatory tests be continued in comparison with the tentative Lichthardt method.

(2) That further collaborative study be made of the tentative permanganate oxidation number.

(3) That methods for the quantitative determination of free mineral acids in vinegar be further studied.

(4) That work on determination of tartrates in vinegar be continued.

(5) That methods for determining sorbitol, and the usefulness of this value in detecting cider vinegar in wine vinegar, be studied.

(6) That the tentative method for starch in salad dressing be adopted as official (first action), and that no further work be done. The wording of the method, after the preliminary preparation, should be the same as that of the method for starch in mustard.

(7) That further study be made of the tentative method for fat in salad dressings, particularly those containing less than 50 per cent fat, so that the method may be made official.

(8) That the efficiency of the preliminary removal of fat in the official method for nitrogen in mayonnaise be studied.

(9) That the tentative method for starch in prepared mustard and mustard flour, as described in the Associate Referee's report, be made official (first action). The wording of the method, after the preliminary preparation, should be the same as that of the method for starch in mayonnaise and salad dressing.

(10) That studies be made of a suitable method for determination of ash in prepared mustard.

(11) That the official method for copper-reducing substances by direct inversion **(33.40)** be dropped (final action).

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* For report of Subcommittee C and action by the Association, see *This Journal*, 31, 54 (1948).

(12) That studies be made of a suitable method for determination of sugars in prepared mustard.

(13) That studies be continued on the determination of volatile oil and other pungent principles in mustard.

(14) That studies be continued on volatile oil in spices.

REPORT ON VINEGAR

By James H. Loughrey (Food and Drug Administration, Federal Security Agency, Boston, Mass.), *Associate Referee*

Of the recommendations approved by Committee C last year (*This Journal*, **30**, 53, 1947), consideration has been given to those on caramel tests and the permanganate number. The Mallory and Love method seems to be less fitted for the detection of caramel in vinegar than are several methods used for its detection in wines and spirituous liquors. These two methods are Mathers test and the present A.O.A.C. confirmatory test (**15.39**), both to be finally confirmed by the use of 2.4-dinitrophenylhydrazine reagent. These tests and the results of collaborative work are the subject of Valaer's report on caramel coloring (p. 178).

Several complaints have been received about false positives when the present tentative method (**33.78**), for caramel in vinegar, is used on cider vinegars. These false tests appear to occur in old cider vinegars. The Associate Referee has obtained one such test on a cider vinegar, three years old, whose authenticity seems to be unquestioned. The two tests reported on by Valaer, on the other hand, seemed to give accurate results. Further study of the tentative method is therefore indicated.

The wording of the method for determining the permanganate oxidation number was changed slightly. The reworded method follows:

REAGENTS

(a) *Sulfuric acid soln.*—1+1.

(b) *Potassium permanganate soln—approx. 1 N.*—It is suggested that the soln be slightly stronger than 1*N*; however, standardization is not necessary. Prepare according to **43.17**, p. 807.

(c) *Sodium thiosulfate—0.5 N.*—Accurately standardized against $K_2Cr_2O_7$. (Method **43.28**, p. 809, should be modified to correspond to the stronger $Na_2S_2O_3$. It has been found that the following quantities of reagents are satisfactory—0.50 gm $K_2Cr_2O_7$, 10 gm KI, 10 ml *conc.* HCl, and 90 ml H_2O.)

(d) *Potassium iodide soln.*—Dissolve 30 g of KI in 100 ml of H_2O, and filter. Do not use unless colorless.

DETERMINATION

Adjust vinegar to 4 g/100 ml acidity as acetic acid. Steam distil 50 ml of adjusted vinegar and collect 50 ml of distillate; distillation should be regulated so that ca 45 ml remain in distilling flask when 50 ml of distillate are collected. (All-glass apparatus is preferable; if not available, cork or rubber stoppers should be covered with

Sn or Al foil). The apparatus used for determination of volatile fatty acids in fish products (24.9, p. 361) is very convenient for this distillation. Keep distillate and reagents at 25°C.

Transfer the 50 ml of distillate to 250 ml glass-stoppered Erlenmeyer flask. Add 10 ml of the H_2SO_4 soln and 25 ml (accurately measured) of the $KMnO_4$ soln. Hold at 25°C., preferably in water bath, for exactly one hour. Then immediately add 20 ml of the KI soln and mix well. Titrate the liberated I with the 0.5 *N* $Na_2S_2O_3$. Conduct a blank determination at the same time, using 50 ml of H_2O, 10 ml of the H_2SO_4 soln and 25 ml of the $KMnO_4$ soln.

The number of ml of the 0.5 *N* $Na_2S_2O_3$ required by the blank less the quantity used in the determination divided by two is the permanganate oxidation number of the vinegar. Report on basis of the adjusted vinegar (4% acid).

The reworded method was sent to collaborators together with four samples of vinegar, to be analyzed according to the proposed method. The composition of the four samples was as follows:

Sample A—Cider vinegar, reduced to 4% acidity.
Sample B—Distilled vinegar, reduced to 4% acidity.
Sample C—Dilute Acetic Acid (4%).
Sample D—75% Cider Vinegar, 25% Dil. Acetic Acid (Acidity of mixture, 4%).

COLLABORATORS' RESULTS

COLLABORATOR	STATION	SAMPLE A	SAMPLE B	SAMPLE C	SAMPLE D
Sidney Kahan	New York	6.10	3.02	0.00	4.56
		6.09	3.06	0.00	4.56
		Av. 6.10	Av. 3.04	Av. 0.00	Av. 4.56
F. M. Garfield	St. Louis	5.70	3.0	0.00	4.2
Robert E. O'Neill	Atlanta	6.01	2.93	0.19	—
		6.06	3.21	0.05	2.61
		Av. 6.04	Av. 3.07	Av. 0.12	
C. G. Cunningham	Boston	5.17	2.56	0.18	4.05
		5.23	2.62	0.19	4.16
		Av. 5.20	Av. 2.59	Av. 0.19	Av. 4.11
A. L. Suslam	Boston	5.26	2.85	0.03	4.01
M. D. Voth	Boston	5.38	2.83	0.06	4.24
J. H. Loughrey	Boston	5.20	2.53	0.15	4.09
		5.27	2.55	0.14	3.99
		Av. 5.24	Av. 2.54	Av. 0.15	Av. 4.04

COLLABORATORS' COMMENTS

Sidney Kahan: **"Concentration of permanganate solution as given is somewhat vague. Suggest that it be made about 31 grams per liter in order to avoid having the blank titrate more than 50 ml. Also suggest that the instructions be amended to include draining the permanganate pipette for a definite length of time, as in iodine number, to secure more uniform results."**

F. M. Garfield: "Amount of KI used should be increased to prevent precipitation of iodine before the titration with the thiosulfate."

The results of the collaborative work are quite promising. Although there are variations between the results obtained by the different collaborators on any one sample, the values obtained for any one type of vinegar are in the same range. The type of reaction involved and the strength of reagents necessary for the method are such that slight variations may be expected. The results on Sample D were very encouraging. This sample consisted of 75% of Sample A and 25% of Sample C. With one exception, the actual results by any one collaborator on Sample D agree very closely with the theoretical value calculated on his figures for A and C.

The Associate Referee wishes to thank the collaborators for their prompt assistance.

RECOMMENDATIONS*

It is recommended—

(1) That the method for the determination of caramel in vinegar **(33.78)** be dropped and that studies be made of other tests for caramel, including the Mathers test, and the test for caramel in wine **(15.39)**, to determine their applicability to the detection of caramel in vinegar.

(2) That the permanganate oxidation method be applied to a number of samples of distilled vinegars of known history to determine its value in differentiating this type of vinegar from a dilute acetic acid.

(3) That the methods for the quantitative determination of tartaric acid and tartrates in vinegar be further studied.

(4) That studies of the determination of tartaric acid and tartrates in vinegar be continued.

No report was given on volatile oil in spices.

REPORT ON STARCH IN PREPARED MUSTARD AND MUSTARD FLOUR

By FREDERICK M. GARFIELD (Food and Drug Administration, Federal Security Agency, St. Louis, Mo.), *Associate Referee*

The determination of added starch in prepared mustard and mustard flour has been the subject of study for a number of years. Several methods have been investigated, and the most satisfactory was found to be a modification of a procedure developed by Sullivan[1] for the estimation of starch in plant materials. The method,[2] when first subjected to collaborative study called for (1) solution of the starch (in the mustard product) in boiling 30% calcium chloride solution; (2) precipitation of the starch from the calcium chloride solution with alcohol to remove soluble interfering reducing substances; (3) a further purification of the starch by solution in water and reprecipitation as a starch-iodine complex; (4) decomposition of the complex with alcoholic sodium hydroxide to recover the starch; (5) hydrolysis of the starch to dextrose by boiling in hydrochloric acid and (6) determination of the resulting dextrose by the Munson-Walker copper reduction method. The starch was calculated using the equation dextrose × 0.9 = starch. Collaborative results were encouraging, but recoveries were somewhat low and erratic.

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* For report of Committee D and action of the Association, see *This Journal*, 31, 54 (1948).
[1] *This Journal*, 18, 621 (1935).
[2] J. T. Field, *Ibid.*, 24, 700 (1941) and 25, 705 (1942).

Further study[3] showed that modifications in the procedure were necessary. It was demonstrated that failure to neutralize the acidity in prepared mustard caused losses as high as 5% in starch recovery. These losses occurred during the initial solution of the starch in the calcium chloride solution, apparently due to partial hydrolysis of the starch by the vinegar present in the prepared mustard. Neutralization of free acidity was made a part of the method. A correction for water introduced by the moisture

RESULTS OF COLLABORATORS

Sample 1.—Prepared Mustard

COLLABORATOR	DATE ANALYZED	STARCH FOUND	RECOVERY	DATE REANALYZED	STARCH FOUND	RECOVERY
		per cent	*per cent*		*per cent*	*per cent*
1	4/ 9/47	4.50	102.0	5/ 9/47	4.32	98.0
		4.34	98.4		4.48	101.6
2	4/16/47	4.30	97.5	5/16/47	4.58	103.9
		4.46	101.1		4.45	100.9
3	4/15/47	4.52	102.5	5/15/47	4.52	102.5
		4.34	98.4		4.39	99.5
3				5/15/47	4.30	97.5
					4.39	99.5
5	4/21/47	4.53	102.7			
		4.46	101.1			
6	4/11/47	4.45	100.9	5/ 6/47	4.32	98.0
		4.39	99.5		4.28	97.0
Averages		4.43			4.40	
Overall average		4.416% or 100.1% recovery				
Standard deviation		±0.088% of starch				
Probable error		0.059% of starch				
Range		4.58−4.28 = 0.30%				

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[3] *Ibid.*, in press.

Sample 2.—Mustard Flour

COLLABORATOR	STARCH FOUND	RECOVERY
	grams	*per cent*
1	0.2117	99.8
	0.2153	101.5
2	0.2102	99.1
	0.2124	100.1
3	0.2196	103.5
	0.2102	99.1
4	0.2074	97.7
	0.2074	97.7
5	0.2102	99.1
	0.2137	100.7
6	0.2102	99.1
	0.2102	99.1
Average	0.2115	99.7
Standard deviation	±0.0033 g	
Probable error	0.0023 g	
Range	0.2196 − 0.2074 = 0.0122 g	

present in the prepared mustard was included for obvious reasons. Minor manipulative changes were made to permit a more convenient method of decomposing the starch-iodine complex. The method as modified then gave good recoveries of starch except for an occasional high result. These high results were due to failure to remove interfering mustard material after solution of the starch in calcium chloride. To accomplish this the method was modified to call for centrifuging the calcium chloride solution digest and filtration through cotton on this partially clarified solution. Unless removed, solid material is carried through the various steps along with the starch and is eventually broken down during the hydrochloric acid hydrolysis to substances capable of reducing Fehling's solution. As a result they are calculated as starch and lead to high recoveries.

PREPARATION OF SAMPLES AND INSTRUCTIONS TO COLLABORATORS

Sample 1.—Prepared Mustard

This sample was made by adding commercial corn starch to a commercially prepared mustard. The starch assayed 84.89% starch (ave. of 6 determinations) by direct acid hydrolysis and subsequent estimation of dextrose by the Munson-Walker method. Starch was calculated as dextrose × 0.9. As prepared, the sample contained 4.41 per cent of 100 per cent starch. Solids and acidity determinations were made by the Associate Referee and reported to the collaborators.

Instructions were to assay the sample by the method given in *This Journal* **30,** 75 (1947) on receipt of the sample, and again in one month. The mustard was to be held at room temperature during this period.

Sample 2.—Mustard Flour

A sample of commercially prepared mustard flour and one of corn starch were sent to collaborators with instructions to weigh 2.0000 g mustard flour and 0.2500 g starch (equivalent to 0.2122 g of 100% starch) and assay by the same procedure.

COLLABORATORS' COMMENTS

H. P. Bennett: "Difficulty was encountered in the resuspension of the starch in water after the first precipitation with 95% alcohol." The following slight change was introduced. "The alcohol was decanted from the starch mass after centrifuging without filtering thru asbestos. Glass beads were added to the bottle after the water was added and vigorous shaking for a few minutes carried. out. This suspended the starch very well and the subsequent extraction of the excess iodine with alkaline alcohol was expedited."

S. D. Fine: "We had some difficulty filtering the $CaCl_2$ solution of the starch thru a pledget of cotton. McNall suggested filtering thru a thin layer of cotton in a funnel, much as in the insoluble solids determination in preserves. This worked much better than the pledget in a funnel.

DISCUSSION

Comments of both collaborators are quite pertinent. The addition of glass beads to aid in breaking up the precipitated starch, as suggested by Bennett, had been tried independently by Joiner and the Associate Referee and found to give good results.

McNall's suggestion of filtering the calcium chloride solution thru a thin layer of cotton is a good one. This filtration is generally quite slow, since the solid material is slimy and readily clogs both paper and cotton.

The results reported by the collaborators are quite good. For mustard flour the average recovery (ave. of 12 determinations) is 99.7 per cent of the starch added, and for prepared mustard (ave. 20 determinations) 100.1 per cent. The standard deviations are not excessive, especially when consideration is given to the number of manipulations required by the method. Even the range (difference between maximum and minimum values) is reasonably good, and the range coefficient (defined as the quotient of the range and the arithmetic mean) does not exceed 7 per cent for either method.

There appears to be no loss of starch in prepared mustard when the mustard is allowed to stand at room temperature for one month. This is shown by the recoveries under Sample 1.

The Associate Referee is indebted to the following members of the Food and Drug Administration field laboratory staff: H. P. Bennett, Kansas City, Mo.; S. D. Fine and F. J. McNall, Cincinnati, Ohio; D. M. Heller and C. R. Joiner, St. Louis, Mo.

RECOMMENDATIONS*

It is recommended that the present tentative method for starch in mustard flour and prepared mustard, with slight modification of wording† be adopted as official, first action, and further work on this subject be discontinued.

REPORT ON STARCH IN MAYONNAISE AND SALAD DRESSING

By SAM D. FINE (Food and Drug Administration, Federal Security Agency, Cincinnati, Ohio), *Associate Referee*

The present tentative method[1] for the determination of starch in mayonnaise and salad dressing was subjected to further study in accordance with the recommendations of the Referee on spices and condiments. A

TABLE 1.—*Mayonnaise containing 3.92% starch.*
(Calculated from weight of air dry starch and acid hydrolysis of starch)
Prepared March 31, 1947

ANALYST	DATE OF ANALYSIS	STARCH	RECOVERY
		per cent	*per cent*
S. D. Fine	4/ 1/47	3.85	98.2
	4/ 1/47	3.88	99.0
	4/ 1/47	3.84	98.0
	5/ 5/47	4.29	109.4
	5/ 5/47	4.04	103.1
F. M. Garfield	4/ 3/47	4.07	103.8
	4/ 3/47	4.09	104.3
	5/ 6/47	4.00	102.0
	5/ 6/47	3.94	100.5
D. M. Heller	4/ 9/47	4.07	103.8
	4/ 9/47	4.17	106.4
	5/ 9/47	3.97	101.3
	5/ 9/47	3.99	101.8
H. C. Van Dame	4/ 4/47	3.71	94.6
	4/ 4/47	3.72	94.9
	5/12/47	3.80	96.9
	5/12/47	3.79	95.7
		Ave. 3.95	100.8

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* For report of Subcommittee C and action by The Association, see *This Journal*, 31, 55 (1948).
† The modifications are reported in *This Journal*, 31, 108 (1948).
[1] *This Journal*, 30, 74 (1947).

previous report[2] had suggested the possibility that losses in starch originally present in mayonnaise might occur on ageing.

EXPERIMENTAL

Two different starch containing mayonnaises were prepared and submitted for collaborative study with instructions to the collaborators to determine starch immediately on receipt and again at the end of a month. The mayonnaises were held at room temperature during this month.

Tables 1 and 2 give the results obtained on the two samples.

TABLE 2.—*Mayonnaise containing 4.10% starch*
(Calculated from weight of air dry starch and acid hydrolysis of starch)
Prepared April 2, 1947

ANALYST	DATE OF ANALYSIS	STARCH	RECOVERY
		per cent	*per cent*
S. D. Fine	4/ 3/47	3.85	93.9
	4/ 3/47	3.93	95.9
	5/ 5/47	4.01	97.8
	5/ 5/47	3.95	96.3
H. D. Van Dame	4/ 4/47	3.77	92.0
	4/ 4/47	3.84	93.7
	5/12/47	3.86	94.1
	5/12/47	3.82	93.2
		Ave. 3.88	94.6

DISCUSSION

The samples submitted for study were prepared by placing a weighed amount of air dry starch in a tared beaker and adding water. The material was heated on a steam bath until a smooth paste was formed. After cooling, egg yolk, spices, sugar, oil, etc., in known amounts were added while the contents were stirred with an electrical stirrer. The final weight was obtained, added water was calculated by difference, and the percentage of starch calculated. Each batch yield was approximately two pounds. The uniformity of the mayonnaise so prepared may not be as perfect as desired or as might be obtained in commercial practice. This may account for the spread obtained in the analytical results.

Consideration must be given to the complex nature of starch in evaluating the results obtained in the quantitative determination of this substance in foods. The precision obtained in the determination of a simple inorganic compound is not possible in the determination of a complex organic material such as starch. It is the belief of the author that the present method gives about the maximum precision obtainable in the estimation of starch in mayonnaise and salad dressing.

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[2] Sam D. Fine, *Ibid.*, **27**, 261 (1944).

The results obtained indicate that at the end of one month there is no detectable loss of the amount of starch originally present in a starch-containing mayonnaise.

SUMMARY

There is no detectable loss of starch from starch-containing mayonnaise, when the mayonnaise is held at room temperature for a month. The precision of the present method is sufficient for the determination of starch in mayonnaise and salad dressing.

RECOMMENDATIONS*

It is recommended—

(1) That the present tentative method be adopted as official, first action, and that the study be dropped.

REPORT ON METALS, OTHER ELEMENTS, AND RESIDUES IN FOODS

By H. J. WICHMANN (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.), *Referee*

Associate Referee's reports were received on copper and DDT. A contributed paper on mercury spray residues on apples was also read at the meeting.[1] The Referee's report will, therefore, be confined to these and related subjects.

COPPER

The Associate Referee reports the experience of his laboratory (one that has probably had more experience with copper methods than many others) with the all-dithizone copper methods. His report summarizes the advantages and disadvantages of each, and concludes with a recommendation for collaborative study of two methods with the greatest sensitivity, one a "two-color" and the other one a "one-color" method. The Referee approves this recommendation.

MERCURY

The Referee became well aware of the peculiar difficulties encountered when the Laug method for mercury was applied to apples, and the implications thereof. Little progress had been made for some years on the determination of mercury until the novel and unorthodox suggestion was made of using selenium, either as a catalyst as in Kjeldahl digestions or as a fixative, the reverse of the official selenium method, in the preparation of the sample. Thus, a rather good beginning of an entirely new approach to the mercury problem has been made, and the prospects are much brighter. The Referee recommends that strenuous efforts be made to perfect the new method of sample preparation for all kinds of organic materials, especially as applied to spray residues. The stripping method so successful in the past on all sorts of spray residues on fruits should be applicable to mercury residues. Other things being equal, the decided decrease in the amount of organic material that must be oxidized after a stripping should make for success.

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* For report of Subcommittee C and action by The Association, see *This Journal*, 31, 55 (1948).

[1] Freda Kunze, "Determination of Mercury on Apples," see *This Journal*, p. 438.

The present dithizone method, for the actual determination of mercury, might be made even more sensitive by the Greenleaf "two-color" dithizone procedure, as developed in connection with the determination of copper. The Referee hopes that interest in the determination of mercury in organic matter will be intensified and that, as a result, more rapid progress will become possible.

DDT

The collaborative results on the determination of DDT reported by the Associate Referee are fairly good, with the two colorimetric methods producing the best results, the methods based on the determination of organic chlorine, either labile or total, being somewhat disappointing. The generally high results for the total chlorine method may stem from inability to successfully separate organic chlorine from inorganic chlorine, or from the insensitivity of the Volhard titration, especially in the presence of incompletely removed natural coloring matter. The labile-chlorine method may suffer from the difficulties just mentioned and, in addition, from the removal of more than one chlorine atom from some varieties of commercial DDT (o′ p′ fraction). The chloride methods are actually in use in all sorts of investigations and, in the case of some of the newer insecticides, are all that are available at the present time. Therefore, it is not expedient to recommend tentative adoption of the color methods and exclusion of consideration for the chloride methods on the basis of these first collaborative results. The Referee approves the Associate Referee's recommendation that the chloride methods be further studied, particularly with respect to the exclusion of inorganic chlorides, and to a more sensitive method for the determination of the chlorine ion. The Referee understands that the electrometric method is good when it works, but that the instrument sometimes gets out of order for some not readily determined reasons. Some suggestions along chemical lines have already been made. The Referee hopes that these will be followed up.

There is not too much information about interferences in the DDT determination, such as those caused by other chlorinated insecticides. Furthermore, no report on the determination of DDT in canned foods is being made. The Referee believes there is no demand for hasty adoption of tentative methods for DDT at the present time, since a new edition of the A.O.A.C. *Methods of Analysis* is not due for two years. All of these reasons impel the Referee to postpone the making of recommendations for tentative adoption of DDT methods for a year, in the hope that some of the uncertainties of the determination in canned, as well as in other, foods will be clarified by studies to be expanded during the year.

BENZENE HEXACHLORIDE

At the present moment there exists a tremendous demand for a sensitive, accurate, and rapid method for the determination of benzene hexachloride ($C_6H_6Cl_6$) existing as spray residue on foods and forage crops, as a contaminant of soils by spray residue or by deliberate addition; in fatty foods and tissues by absorption; in biological material of all kinds, and in or on foods in general. The strong suspicion that commercial benzene hexachloride (BHC), or some of its impurities, can cause unpleasant flavors in foods, directly or by starting undesirable physiological processes, has increased this demand. The infra-red method, applicable to the insecticide itself, cannot be applied to the small amounts present in spray residues, contaminated as they are with all sorts of interferences, insecticidal and non-insecticidal. The recoveries with the colorimetric method, mentioned last year as being in the process of development, have been 80 to 90 per cent, and in a few instances, 95 per cent. The losses appear to occur just prior to or during the nitration of the trichlorbenzene derived from the benzene hexachloride. Also too little is known about interferences, and this method is not ready for publication. Another method based on the measurement of certain absorption peaks in the ultra-violet absorption spectrum of 1-2-4 trichlorbenzene (derived from benzene-hexachloride isomers in greater proportion than the other two possible trichlorbenzenes) has come to the attention of the Referee.[2] It is understood that it leaves something to be desired in the way of sensitivity and accuracy, and needs further development. A biological method,[3] with the house fly as the test animal, has been used with some degree of success for the determination principally of the gamma isomer of benzene hexachloride in biological material. All of these methods need further development and study of the effect of interferences before publication. This leaves the chlorine methods as the last recourse, but any recommendation for their use can be made only with a degree of hesitation and reserve in view of the numerous other chlorine-containing insecticides, hormones, or herbicides in use or in prospect. Within the next year a benzene hexachloride method may be developed, but the appointment of an Associate Referee would now be premature.

CHLORDANE AND TOXAPHENE

No definite methods for the determination of chlordane or toxaphene, other than those based on their chlorine content, has come to the attention of the Referee. The chlorine methods, of course, lack specificity. Suggestions for methods of analysis for these products in the form of spray residues, or in biological materials of all kinds, from the manufacturers of these products, would be much appreciated. Undoubtedly, the Association will have to give attention to these products and their residues as soon as it can, but this can hardly be done before the determinations of DDT and benzene hexachloride are out of the way.

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[2] Private Communication from B. Davidow, Pharm. Div., Food and Drug Administration.
[3] Private Communication from Edwin P. Laug, Pharm. Division, Food and Drug Administration

Hexaethyltetraphosphate (HETP) and diethylparanitrophenylthiophosphate (AATP)

Two new phosphorus-containing insecticides, under these names, have come to the attention of the Referee. They are said to be very toxic to both insects and warm-blooded animals. Hexaethyltetraphosphate, known to the Germans as "Bladan," is useful for the treatment of aphids and the mite tribe of insects in general. It is generally used as an adjunct to DDT. It is readily altered and loses its effectiveness as an insecticide in 24 hours. Apparently the decomposition products (not exactly known at present) do not possess any serious degree of toxicity for warm blooded animals. While HETP may have serious effects on careless sprayer crews, it is not expected to build up toxic spray residues. Hardin and MacIntire[4] report that hexaethyltetraphosphoric, triethylphosphoric, or monoethylphosphoric acids all require long treatment with aqua regia at the boiling point before they will hydrolyze completely to orthophosphoric acid. Whether reaction with alkalies is more expedient or rapid remains to be determined. A method for the determination of HETP and its decomposition products may be possible but not easy or rapid. Since its use is not expected to result in toxic residues, the Referee believes the Association can ignore it, at least for the present.

The Referee understands that diethylparaphenylthiophosphate (AATP) will do what HETP does and be effective for the codling moth besides. Hence it may be expected to have wider application. Fortunately the manufacturers of this compound have supplied a micro method[5] for its determination simultaneously with its commercial introduction. Hence there need be no time lost in development of a method. Perhaps the Referee will be in a position to assess the extent of the commercial usage of AATP by the next meeting and be prepared to recommend collaboration on a method for its determination.

RECOMMENDATIONS*

It is recommended—

(1) That the work on the determination of cadmium, copper, mercury, and zinc be continued.

(2) That the determination of DDT in canned foods and in foods in general be studied further.

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[4] *This Journal*, present number, p. 400.

[5] Unpublished; obtainable from American Cyanamide Co., Stamford, Conn.

* For report of Subcommittee C and action by The Association, see *This Journal*, 31, 52 (1948).

REPORT ON COPPER IN FOODS

By G. H. **Bendix** (Continental Can Company, Inc., Chicago, Ill.), *Associate Referee*

Collaborative work on copper in foods was last conducted by Greenleaf (1) in 1945, at which time he described results obtained by a combined dithizone-carbamate method. Greenleaf recommended that the dithizone-carbamate method be adopted as tentative, but he also pointed out that serious consideration should be given to the development of an all-dithizone method. In recent years several all-dithizone methods have appeared in the literature (2, 3, 4). The earliest of these is that of Bendix and Grabenstetter, which is a single color method, *i.e.* the violet copper dithizonate is separated from the green uncombined dithizone before determining the optical density. The second method, that of Morrison and Paige, is essentially the same as that of Bendix and Grabenstetter except that the excess dithizone is not removed, the spectrophotometric measurements being made on a two-color system. The most recent publication, that of Greenleaf, is also a "two-color" method and avoids objectionable features of the two other methods just mentioned.

In his 1946 report on metals in foods Wichmann (5) commented on the advantages of the Greenleaf "two-color" method and recommended it to the Associate Referee.

The method of Bendix and Grabenstetter is subject to two criticisms: (a) The extraction of copper, being carried out at a *p*H of 2.3 and in the presence of a high concentration of dissolved salts, requires an excessively long shaking time of 10 minutes and thereby necessitates a mechanical shaker unless only a few determinations are to be made. (b) Removal of the excess dithizone by extraction with dilute ammonia subjects the violet keto copper dithizonate to conversion to the yellow enol tautomer. This fact has been pointed out by Sandell (6) and by Morrison and Paige. The latter authors avoid the second of these objections by omitting the extraction with ammonia, thereby making the method a "two-color" method. This change, although it also simplifies the laboratory manipulations, is accompanied by a very appreciable decrease in sensitivity.

The Greenleaf method avoids both of the above mentioned objections to the Bendix-Grabenstetter method and does so without a sacrifice of sensitivity which characterizes Morrison's method. This accomplishment, however, was not achieved without a sacrifice of the simplicity common to the other two methods.

Although collaborative work has not been conducted during the past year, work has been carried out in the Associate Referee's laboratories for the purpose of comparing, one with the others, the all-dithizone methods of Bendix-Grabenstetter, Morrison-Paige, and Greenleaf. Table 1 shows the results obtained.

TABLE 1.—*Comparison of all-dithizone methods for copper in foods*

SAMPLE	BENDIX-GRABENSTETTER	MORRISON-PAIGE	GREENLEAF
	p.p.m.	*p.p.m.*	*p.p.m.*
Dried Peas	34.9	31.5	30.9
	35.2	32.4	
Dried Corn	14.2	14.5	14.0
	14.0	13.8	
Chocolate Ovaltine	14.7	—*	13.8
Pea brine A (Canned)	1.09	1.10	—*
	1.09	1.10	
Pea brine B (Canned)	1.15	1.15	—*
	1.15	1.15	

* No analysis made.

DISCUSSION OF RESULTS

The results of Table 1 show no significant difference in the results obtained by the three methods and indicate that the objections to the Bendix method (conversion to enol tautomer) and the Morrison method (lack of sensitivity) are not as significant as might be expected. This fact may not be capable of duplication by other laboratories, since it must be admitted that the Associate Referee's laboratory has extensive experience with the Bendix method and may have avoided the objectionable features by carefully controlling the conditions during the extraction of excess dithizone with dilute ammonia.

The various advantages and disadvantages of the three methods are given in the summary below, from which it may be noted each method has certain desirable characteristics. The method of Greenleaf, however, appears to more nearly approach the ideal of a referee method.

A COMPARISON OF THE CHARACTERISTICS OF ALL-DITHIZONE METHODS FOR COPPER IN FOODS

Specificity.—Little difference between the three methods. All methods probably satisfactory for most food products. All methods subject to interference by excessive concentrations of bismuth and noble metals.

Sensitivity.—Morrison method appreciably inferior to those of Bendix and Greenleaf, the latter two having a very high sensitivity.

Simplicity.—Greenleaf method considerably more complicated with respect to technique and reagents required.

Theoretical Soundness.—Greenleaf method is best with the Morrison method a close second best. Bendix method open to question with respect to the one color technique.

The Associate Referee is indebted to Mr. W. C. Stammer and his associate, Mr. N. H. Strodtz, for conducting the analyses reported above and for their comments relative to the methods under consideration.

RECOMMENDATIONS*

It is recommended that the "two-color" method of Greenleaf and the "one-color" method of Bendix and Grabenstetter be submitted to collaborative study.

LITERATURE CITED

(1) GREENLEAF, C. A., *This Journal*, **28**, 269 (1945)

(2) BENDIX, G. H., and GRABENSTETTER, D., *Ind. Eng. Chem., Anal. Ed.*, **15**, 649 (1943).

(3) MORRISON, S. L. and PAIGE, H. L., *Ibid.*, **18**, 211 (1946).

(4) GREENLEAF, C. A., *This Journal*, **30**, 51 (1947).

(5) WICHMANN, H. U., *This Journal*, **30**, 451 (1947).

(6) SANDELL, E. B., "Colorimetric Determination of Traces of Metals," pp. 84, 218, *Interscience Publishers*, New York, 1944.

REPORT ON DDT IN FOODS

By R. H. CARTER (Agricultural Research Administration,
Bureau of Entomology and Plant Quarantine,
Beltsville, Md.), *Associate Referee*

In 1946 the Associate Referee presented a report (1) reviewing the methods which had been recommended or presented for the determination of DDT in insecticide residues and in animal products. Methods for the preparation of samples were discussed briefly. No samples were sent out for collaborative testing.

In 1947 an apple-strip solution containing a known added amount of DDT was prepared, and identical samples were submitted to a number of collaborators who had expressed a willingness to take part in this program. This solution was prepared as follows:

Samples of apples weighing approximately 2 kg. were placed in 2½-gallon wide-mouth pickle jars, 500 ml of benzene was added to each, and the jars were rotated sidewise in a tumbling machine at approximately 4 r.p.m. for 30 minutes. This method of stripping does not bruise the apples excessively, but imparts enough rolling motion with a slight abrasive action to insure thorough wetting of the surfaces and solution of the wax and DDT. At the end of the 30-minute period the machine was stopped, the samples were allowed to drain for a few minutes, and the benzene solution was decanted, the amount measured and then calculated as an aliquant of the original 500 ml. Approximately 10 liters of this strip solution was made. No DDT-free apples were available; so a number of analyses were made to determine the DDT content of this solution. To this solution was then added enough pure *p, p'*-DDT and *o, p'*-DDT in the ratio of 3 to 1 to make a total content of 17.12 mg. per 500 ml; the volume also was adjusted so that each 500 ml represented 2 kg. of apples. Seventeen samples of 500 ml each were then sent out to the collaborators. The method of analysis to be used was left to the choice of the analyst. Unfortunately two samples were lost in transit.

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* For report of Subcommittee C and action by The Association see *This Journal*, **31**, 52 (1948).

Results of the analyses by four methods with modifications are shown in Table 1.

TABLE 1.—*Results of analyses of apple-strip solutions for DDT by different methods (DDT present, 8.6 p.p.m.)*[1]

COLLABORATOR	LABILE-CHLORINE METHOD[2]	TOTAL-CHLORINE METHOD[3]	COLORIMETRIC METHODS	
			SCHECHTER-HALLER	STIFF-CASTILLO
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*
A	—	8.7*	—	—
B	—	—	8.5	—
C	—	—	—	8.3
D	9.7	7.1	7.8	7.8
E	—	—	—	8.3
F	15.0	10.7		
G	—	8.4 8.2*		
H	14.9	12.4		—
I	—	—	—	9.1
J	—	9.9	—	8.7
K	—	10.9 8.2*	8.6	
L	10.4	11.1	—	
M	10.6	8.6	—	
N	8.8	8.3	—	8.4
Average	11.6	9.5	8.3	—

[1] Results reported are averages of 2 to 5 determinations.

[2] Collaborator N used procedure 1 B, all others used 1 A, All used Volhard titration.

[3] Collaborators A, G, and N used procedure 2 B, all others used 2 A. Asterisk (*) indicates determinations by electrometric titration; all others by Volhard titration.

A summary of the results reported by the four methods is given in Table 2.

TABLE 2.—*Summary of the results reported in analysis of collaborative apple strip solution No. 1, containing 8.6 p.p.m. of DDT*

METHOD OF ANALYSIS	NUMBER OF COLLABORATORS	DDT REPORTED (P.P.M.)		
		MINIMUM	MAXIMUM	AVERAGE
Labile chlorine	6	8.8	15.0	11.6
Total chlorine	10	7.1	12.4	9.5
Schechter-Haller colorimetric	3	7.8	8.6	8.3
Stiff-Castillo colorimetric	5	7.8	9.1	8.4

The collaborators were as follows:

Food and Drug Administration, Federal Security Agency:
Robert H. Dick, L. W. Ferris, Sam D. Fine, Curtis R. Joiner, Harold F. O'Keefe, C. D. Schiffman, Shirley M. Walden, Louis C. Wiess.

Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture:
P. E. Hubanks, L. Koblitsky, H. D. Mann, W. E. Westlake.

California State Department of Agriculture:
J. B. LaClair.

Maine Agricultural Experiment Station:
A. Stanley Getchell.

DISCUSSION

Most of the results reported by the labile-chlorine method were obtained by following the procedure (referred to as 1A) recommended by Wichmann *et al.* (2), in which 350 ml. of the benzene strip solution is treated with 10 ml of 2 *N* alcoholic potassium hydroxide, 250 ml of the benzene solution is then distilled off, the remainder of the solution is refluxed for 30 minutes, and the chloride is determined by standard procedures.

In another procedure (2) (referred to as 1B) for labile chlorine the benzene is distilled off and then 1 *N* alcoholic alkali is added and the solution is refluxed for 10 minutes. The latter procedure appears to give much better results, but even 1 *N* alcoholic alkali gives results considerably too high on some commercial samples of DDT.

The labile-chlorine method has been reported by a number of investigators as being the simplest and easiest for the determination of DDT spray residues on fruit. It is apparently in extensive use in some of the principal apple-producing regions.

Some of the determinations of total organic chlorine were made by the method recommended by Wichmann *et al.* (2). In this procedure (referred to as 2A) the benzene strip solution is refluxed with metallic sodium and a small amount of isopropanol. In another procedure (referred to as 2B) benzene is distilled off on a steam bath and the residue is then refluxed with 25 to 50 ml of isopropanol and metallic sodium. The results reported by this procedure generally appear to agree with the known amount much better than do the results by the former procedure.

Determinations of both labile chlorine and total organic chlorine are rapid and well adapted for routine work. The latter are more accurate for small samples because of the larger amount of chlorine to be determined.

High results in the analysis of the strip solution were reported in many determinations by the labile-chlorine method and to a lesser extent by the total-chlorine method.

An outstanding difficulty in the chlorine determinations is the lack of sensitivity of the indicator, so that the analysts are unable to see the end point in the Volhard back titration with thiocyanate. This is especially true in colored solutions. Electrometric titration apparatus obviates this difficulty, but trouble has been reported by some operators in using such apparatus.

The colorimetric methods developed by Schechter *et al.* as recommended by Wichmann *et al.* (2), and by those of Stiff and Castillo as recommended by Claborn (3), appeared to give consistent results in the analysis of the standard strip solution. Measurements of the color developed were reported as being made by neutral wedge photometers, photoelectric photometers equipped with filters, and spectrophotometers. These methods are of course subject to some interferences, but in general they can be regarded as confirmatory for the presence of DDT.

RECOMMENDATIONS*

(1) It is recommended that a thorough study of the labile-chlorine method be made to determine the optimum conditions, time, temperature, strength of alcoholic alkali, and other factors, including the kinds of agricultural products, such as fruit, vegetables, forage crops, animal products, and soil, to which it is applicable.

(2) It is recommended that a study of the total-chlorine method be continued to determine optimum conditions, such as time, amount of benzene to be left in the flask during refluxing, and other factors, and of the kinds of agricultural products to which it is applicable.

(3) It is recommended that an investigation be made of other methods for the determination of small amounts of chloride.

(4) It is recommended that an investigation be made to determine other insecticide residues that may interfere with the Schechter-Haller and the Stiff-Castillo colorimetric procedures.

LITERATURE CITED

(1) CARTER, R. H., *This Journal,* **30,** 456 (1947).

(2) WICHMANN, H. J., PATTERSON, W. I., CLIFFORD, P. A., KLEIN, A. K., and CLABORN, H. V., *Ibid.,* **29,** 188 (1946).

(3) CLABORN, H. V., *Ibid.,* **29,** 330 (1946).

No reports were given on cadmium, zinc, mercury, or DDT in canned foods.

REPORT ON OILS, FATS, AND WAXES

By J. FITELSON (Food and Drug Administration, Federal
Security Agency, New York, N. Y.), *Referee*

The Associate Referees have made some progress in their studies, but no formal reports will be submitted. The chromatographic technique for purification of unsaponifiable matter[1] has been studied as an alternative method to the somewhat lengthy official S.P.A. washing procedure (**31.40**). Results show very close agreement between the two methods, and collaborative studies are planned for the coming year.

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* For report of Subcommittee C and action by The Association, see *This Journal,* **31,** 51 (1948).

[1] N. D. Sylvester, A. N. Ainsworth, and E. B. Hughes, *Analyst,* **70,** 295 (1945).

The tentative modified Bellier Test (**31.47–31.48**) has been applied to mixtures of peanut oil and other edible vegetable oils. Preliminary results indicate that an approximately quantitative relationship exists between the turbidity temperature and the peanut oil content. The Associate Referee plans to continue studies in this field.

The recently appointed Associate Referee on antioxidants has reviewed the extensive literature on this subject and has also initiated a survey of the field to ascertain the nature of the most commonly used antioxidants. In view of the large number of compounds used for this purpose, it is planned to restrict the initial studies to the most widely used antioxidants.

RECOMMENDATIONS*

It is recommended:

(1) That the official S.P.A. method for unsaponifiable matter (sec. **31.40**) be made official, final action.

(2) That the chromatographic purification of the unsaponifiable matter (1) be studied collaboratively.

(3) That the F.A.C. method for unsaponifiable matter (**31.37–31.39**) be deleted (final action).

(4) That the official, first action, method for squalene (**31.41–31.43**) be made official, final action.

(5) That studies on methods for determining the stability of fats be discontinued for the present.

(6) That studies on methods for the estimation of peanut oil be continued.

(7) That studies on methods for the determination of antioxodants in oils be continued.

No reports were given on unsaponifiable matters, peanut oil, stability of fats, or antioxidants; see Referee report on oils, fats, and waxes.

REPORT ON SOILS AND LIMING MATERIALS

By W. H. MacIntire (University of Tennessee Agricultural Experiment Station, Knoxville), *Referee*

The Referee has functioned mainly in suggestive capacity in obtaining the digests and contributions from the Associate Referees whose reports will be tendered individually. The one exception is the work stipulated in recommendation (5), "That the analytical technique previously proposed for ignition of charge and distillation of fluorine therefrom be studied collaboratively." (*This Journal*, **30**, 43).

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* For report of Subcommittee C and action of the Association, see *This Journal*, **31**, 53 (1948).

A study of that technique resulted in findings to the effect that the prescribed extended calcination of the charge of soil and calcium peroxide at 900°C. is unnecessary and its deletion will be recommended. It was found also that substitution of calcium hydroxide for calcium peroxide is not admissible in calcinations at 900°C., unless the proportion of the hydroxide to soil be at least 10 to 1. The substitution of calcium hydroxide in 1 to 1 proportion was found effective, however, provided the prefatory calcinations of the charges of soil plus calcium hydroxide are conducted at 500°C. for periods in the range of from 5 to 60 minutes.

RECOMMENDATIONS*

It is recommended—

Soils:

(1) That the studies on the "combination dithizone-spectrographic method," and on the polarographic procedure for the determination of zinc in soils, be continued.

(2) That the study of the determination of copper in soils be continued.

(3) That the utilization of carmin as an indicator in the determination of boron content of soils be studied further, and that p-nitrobenzeneazo-1, 8-dihydroxynaphthalene-3, 6-disulfonic acid, or "chromotrope-B," be studied as a reagent suitable for the determination of boron on soils.

(4) That further studies of *p*H in soils of the arid and semi-arid regions be based upon soil systems of moisture content representative of air-dry soil.

(5) That the analytical technique previously proposed for ignition of charge and distillation of fluorine therefrom be studied collaboratively.

(6) That the present wording "at 900° for 30 minutes," under Fluorine (p. 13, section **1.32**, line 3) be made to read "at 500° for 30 minutes."

(7) That a study be made as to the adequacy of $Ca(OH)_2$ as a fixative for fluorine in soil charges of 1 to 1 proportion with calcination at 500°C. in 5 to 60 minute periods.

(8) That the direct distillation of *unignited* soil with H_2SO_4 at 165°C., with sequential distillation of an aliquot at 135°C., be studied collaboratively.

(9) That the "2-point" titration procedure for the determination of exchangeable H in soils be studied further, in relation to liming practice.

(10) That the survey and comparisons of methods for the determination of phosphorus (a) that fraction in "available" state and (b) the proportions of organic-inorganic forms therein (*This Journal*, **30**, 43), be continued.

(11) That the survey and comparisons of methods for the determination of "exchangeable" K in soils (*This Journal*, **30**, 44), be continued.

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* For report of Subcommittee A and action of the Association, see *This Journal*, **31**, 43 (1948).

Liming materials:

(12) That the direct titration against bromcresol green (Method II), *This Journal*, **30,** 297 (1947) be adopted as optional for the determination of the neutralization value of blast furnace slags.

(13) That the tentative procedures in "Liming Materials" be annotated by the statement, "without correction for sulfide content."

(14) That correction for sulfide sulfur content be studied further.

REPORT ON ZINC AND COPPER IN SOILS

By LEWIS H. ROGERS (Department of Soils, Florida Agricultural Experiment Station, Gainesville, Fla.),
Associate Referee

Additional experience has been obtained with the dithizone-spectrographic procedure for the determination of zinc extracted from soils by various extractants. Details are presented below.

The procedure of Sandell[1] has been adapted for use with soil extracts. Reagents are prepared essentially as given by Sandell and also in section 12.24, page 123, *Methods of Analysis*, sixth edition. In addition a standard cadmium solution containing 200 gamma cadmium per ml is prepared; further dilutions are prepared daily. Also a cadmium-dithizonate standard in CCl_4 containing 1 gamma cadmium per ml is prepared.

The soils are extracted with ammonium acetate or other extracting agent in the conventional manner. The leachate is evaporated to dryness, organic matter destroyed with 30% hydrogen peroxide, the residue taken up in a minimum of dilute hydrochloric acid, the solution diluted to 30 ml and an aliquot transferred to a 60 ml separatory funnel. Five ml of 10% sodium citrate solution are added, several drops of purified phenol red indicator solution introduced, and the solution titrated with dilute (1:3) ammonium hydroxide to about *p*H 8, and an excess of 0.5 ml is added. This is extracted with successive portions of 0.01% dithizone in carbon tetrachloride until no further color change is obtained with the dithizone, then one additional extraction is made. Two ml of standard cadmium dithizonate solution are added to the extracted dithizonates, evaporated to dryness on a hot plate at low temperature while air is blown into the beaker to hasten evaporation. The residue is taken up with a minimum of redistilled chloroform and transferred with a micro dropper to a graphite electrode which is heated in a small metal block resting on a hot plate. Graphite electrodes, ¼in.×1 in., with a shallow crater drilled in the lower electrode and upper electrodes pointed in a pencil sharpener, are used.

Spectrograms were taken on a quartz spectrograph with a slit width of 40 microns. The image of the D.C. arc was focused on the collimator with a lens located at the slit of the spectrograph. A rotating step sector with 1:1.5 ratio was used for plate calibration. An iron spectrum was made on each plate with the step sector running; preliminary and final plate calibration curves were plotted as described by Churchill.[2] Background and line intensities were determined from the plate calibration curves in the conventional manner. Eastman process plates, developed in D-19 at 70°F. for 3 minutes and fixed in F-10 were used. Plates were evaluated with a non-recording microphotometer. An analytical working curve was prepared from several independent determinations of known amounts of zinc, with constant amounts of cadmium as internal standard. Working curves were prepared using the zinc line at 3282 A. with the cadmium line at 3261 A. as internal standard.

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[1] "Colorimetric Determination of Traces of Metals," Interscience Publishers, p. 458 (1944).

Preliminary work has shown that with this procedure it is feasible to determine copper and zinc simultaneouly, since copper is concentrated by dithizone under the same conditions as zinc and gives suitable spectrum lines which can be conveniently measured simultaneously with zinc.

It is recommended* that the study be continued.

PRELIMINARY REPORT UPON THE COLLABORATIVE WORK ON THE EXCHANGEABLE POTASSIUM IN SOILS

By IVAN E. MILES (North Carolina Department of Agriculture, Raleigh, N. C.), *Associate Referee*

Realizing that there was some variance of opinion in regards to methods ordinarily used in determining exchangeable potassium, the officials of *A.O.A.C.* have this year initiated some work in an effort to secure a more complete picture of this situation. Several leading laboratories of the country were circularized as to their willingness to assist with a collaborative study of exchangeable potassium. Every worker contacted very graciously agreed to assist.

Subsequently, soils samples with extreme variance in texture, reaction, organic matter, and exchangeable potassium were collected. Two soils came from Arizona, one from New York, one from Tennessee, one from the Piedmont area of North Carolina, and one from the Coastal Plain area of North Carolina.

These soils were air-dried, screened, and mixed thoroughly, and identical samples sent to all collaborators: Brown, Irving G., in Reitemier's Laboratory, U.S.D.A. Caster, A. B., in McGeorge's Laboratory, University of Arizona. Capp, L. C., in Fudge's Laboratory, Texas Agricultural and Mechanical College. Lee, C. K., in Bray's Laboratory, University of Illinois. Mehlich, A., in Research Laboratory, N. C. State College. Piland, Rodney, in Research Service Laboratory, N. C. State College. York, E. T.,

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[2] *Ind. Eng. Chem., Anal. Ed.*, 16, 653 (1944).

* For the report of Subcommittee A and action of The Association, see *This Journal*, 31, 43 (1948).

TABLE 1.—*Potassium collaborative results*

All Data Reported as m.e. of Potassium per 100 grams of Soil

SOILS	COLLABORATORS												
	A[1]	B[1]	C[1]	D[1]	E[2]	F[3]	G[4]	H[5]	C-1[6]	AVE.	C-2[7]	E-1[8]	AVE.
1	1.432	1.055	1.064	1.210	1.270	1.019	1.043	1.469	1.120	1.187	1.025	1.440	1.233
2	3.829	3.727	4.161	3.480	3.380	3.599	3.696	4.636	4.110	3.846	3.080	3.960	3.520
3	.247	.120	.173	.155	.161	.117	.131	.246	.169	.169	.165	.190	.178
4	.205	.106	.128	.180	.147	.069	.109	.221	.118	.143	.133	.117	.125
5	.196	.061	.095	.107	.124	.043	.071	.198	.091	.110	.097	.114	.106
6	.286	.142	.176	.179	.197	.067	.146	.263	.190	.183	.165	.156	.161
Ave.	1.033	.869	.966	.885	.880	.819	.866	1.172	.966		.778	.996	

[1] Ammonium acetate—Volumetric—Potassium Permanganate—U S.D A. Circular 757 (1947).
[2] Ammonium acetate—Volumetric—Ceric Sulphate—*Ind. Eng Chem. Anal. Ed.*, 10, 652 (1938).
[3] Ammonium acetate—Gravimetric—A.O.A C. and *Ind. Eng. Chem , Anal. Ed.*, 9, 136 (1937).
[4] Ammonium acetate—Colorimetric—Nitroso-R-Salt—*Soil Science Soc. Proc.*, 9, 56–60 (1944).
[5] Ammonium acetate—Colorimetric—Nitroso-R-Salt—*Soil Science*, Vol. 59, 25–37 (1945).
[6] Ammonium acetate—Turbidimetric—(After removing excess ammonium acetate) Mimeograph Directions, Ill. Agri. Exp. Sta. (1942).
[7] Sodium nitrate—Turbidimetric—Mimeograph Directions, Ill. Agri Exp Sta (1942).
[8] Barium-chloride—Triethanol amine *N*—*Soil Science Soc. Proc.*, Vol. 9 56–60 (1944).

in Peech's Laboratory, Cornell University, New York. Robinson, Brooks, in MacIntire's and Shaw's Laboratory, University of Tennessee.

The collaborators were requested to use whatever method they normally use, but if the method used is other than the ammonium acetate method, to use the ammonium acetate method also for comparative purposes.

The methods used by the collaborators can be divided into two general categories, namely: 1, extraction and 2, leaching. In the first method, extraction was made with ammonium acetate and the K precipitated as sodium cobalti-nitrite in all cases. Most collaborators used an extracting procedure similar to that described in U.S.D.A. Circular 757. Two investigators used a leaching technique. After precipitating with sodium cobalti-nitrite, four investigators determined K volumetrically with potassium permanganate, whereas one investigator used ceric sulphate procedure. Two investigators determined the K colorimetrically, using nitroso-R-salt, and in one case the K was determined turbidimetrically. One investigator precipitated the K with sodium cobalti-nitrite and determined the K gravimetrically.

From the results obtained (see Table 1) it would seem that any one of the several methods used might be satisfactory in the high range, but there is very considerable variance in the lower or critical range. This would seem to indicate that further work is needed on methods.

Therefore, the writer would recommend* that the work be continued, enlarging it to include other workers. Furthermore, it now seems advisable to have all collaborators use both a leaching and extracting method, and determine K by a volumetric, colorimetric, turbidimetric, or flame photometry procedure.

The writer gratefully acknowledges and appreciates the fine work done, and the spirit of helpful cooperation manifested by all collaborators throughout the study.

No reports were given on hydrogen-ion concentration of soils, boron and fluorine, exchangeable calcium and magnesium, exchangeable hydrogen, or phosphorus in soils. For report on boron under "Plants," see p. 284.

REPORT ON INSECTICIDES AND FUNGICIDES

By J. J. T. Graham, Chemist (Production and Marketing Administration, Livestock Branch, Beltsville, Md.), *Referee*

Consideration of methods of analysis for insecticides, fungicides, and other economic poisons during 1947 was limited to a study of those applicable to rodenticides, products containing DDT, and a preliminary investigation of methods for pyrethrins in pyrethrum flowers. Because of pressure of their official duties, the Associate Referees on fluorine and nicotine were unable to study methods for determination of these ingredients. The General Referee has examined the reports of the Associate Referees on rodenticides and DDT and concurs in their recommendations.

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* For report of Subcommittee A and action by the Association, see *This Journal*, 31, 43 (1948).

METHODS FOR THE DETERMINATION OF PYRETHRINS IN PYRETHRUM FLOWERS

Considerable disagreement with methods for the determination of pyrethrins in pyrethrum flowers has arisen between growers of pyrethrum and the purchasers of the flowers in this country, because results for pyrethrin content obtained in the country of origin have differed materially from those obtained in some laboratories in this country. This difference is principally due to the fact that the pyrethrum growers made their analyses by the official mercury reduction method, while the analyses made for the purchasers were in most instances made by the Seil method.[1]

In order to improve this situation, the Imperial Institute of Great Britain sent representatives to this country, in the latter part of 1946, to solicit cooperation of American chemists in a world-wide study of methods for determination of pyrethrins, with a view to adoption of a uniform procedure for this determination.

As a result of this solicitation, the Chemical Analysis Committee of the National Association of Insecticide and Disinfectant Manufacturers promised the cooperation of that organization; and your Referee promised to solicit the help of official chemists.

The Consultative Committee on Insecticide Materials of Vegetable Origin, of the Imperial Institute, planned the investigation, and it appeared desirable to conduct a preliminary study on variations of the methods in order to decide on the best procedures to be followed in the final tests. A number of modifications of the mercury reduction method were suggested and studied.

The Referee assisted in this preliminary work and further assisted by reviewing and commenting on the revised procedures. A recent letter from Dr. G. T. Bray of the Imperial Institute states that their committee has decided to study the mercury reduction method, the Seil method, and the Ripert method. The latter method was included because it is the principal method used in France.

It is not yet possible to state when the samples will be ready for distribution. However, those chemists who have signified their desire to assist in the work will be kept informed of developments in connection with the investigation. If there are other members of the Association who desire to volunteer their assistance in this work, the Referee will be glad to have their names.

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[1] *Soap*, 10, 89 (1934).

REPORT ON RODENTICIDES

By J. W. Elmore, (State Department of Agriculture,
Sacramento, Calif.), *Associate Referee*

Methods of analysis of rodenticides containing 1080 (sodium fluoroacetate) and Antu (alpha naphthyl thiourea) have been under consideration.

Reactions specific for 1080 have not come to the writer's attention. Total fluorine can be determined in this compound by fusion with sodium peroxide in a Parr bomb followed by precipitation of lead chlorofluoride, as described in the official procedure for fluorine in insecticides. (*Methods of Analysis*, Sixth Edition, page 58 et seq., sec. **6.18, 6.19**). Analysis of a sample of technical 1080 by this method, gave 93.75 per cent. Since determination of total fluorine in grain poisoned with 1080 involves ashing with lime or some other fixative, it was thought possible that some fluorine might be lost during ignition, due to a reaction similar to that which occurs on heating calcium acetate giving acetone and calcium carbonate:

$$(CH_3COO)_2Ca = CH_3COCH_3 + CaCO_3$$

In the case of sodium fluoroacetate, this might be:

$$2\ CH_2FCOONa = CH_2FCOCH_2F + Na_2CO_3$$

However, no evidence of this reaction has been observed. Possibly, fluorine is replaced by the hydroxyl group with the formation of glycollic acid and sodium fluoride similarly to the reaction which takes place when monochloracetic acid is heated with water or alkalies. (*Richter Organic Chemistry*, 1944 Edition, Vol. 1, page 334). Analysis of the above sample of 1080 by drying with lime hydrate slurry and distillation of the ash with perchloric acid gave 93.70 per cent of 1080, of which compares well with results by Parr bomb.

Grains poisoned with 1080 may be analyzed for total fluorine by the general method for fluorine in foods (*Methods of Analysis*, Sixth Edition, page 445, sections **29.24–29.28** incl.) and the result calculated to sodium fluoroacetate. Results so obtained are somewhat high because of the fluorine originally present in the grain, or possibly due to contamination with sodium fluosilicate used for repelling rats and mice in warehouses. Since baits may contain as little as 0.05 per cent of 1080 (corresponding to 0.0095 per cent of fluorine), a considerable error may thus be introduced. Results of analyses of corn and wheat listed by Clifford (*This Journal*, **28**, 278, 1945) indicate about 1 part per million fluorine is to be expected in grains; but local samples analyzed by the writer were considerably higher than this. Further work should be done along this line.

Total combined fluorine in 1080 poisoned grain may be determined on the finely ground sample by weighing 4 grams into a platinum dish and continuing the analysis as directed in the general method (section **29.26 (a)**) beginning "add 25 ml of the $Ca(OH)_2$ suspension."

The solubility of Antu (alpha naphthyl thiourea) in several solvents was determined.

Approximate solubility of Antu, grams per 100 ml solution

SOLVENT	25°C.	100°C.
Acetone	2.43	—
Acetonitrile	1.18	—
Chloroform	0.63	—
1,4-Dioxane	6	30
Ethyl alcohol	0.70	—
Ethyl ether	0.18	—
Petroleum ether	0.001	—
Triethylene glycol	8.6	16
Water	0.006	—

The pure compound contains nitrogen (13.86 per cent) and sulphur (15.84 per cent). Examination of commercial products received to date has shown a higher percentage of Antu calculated from sulphur than from nitrogen. Representative analyses by J. B. LaClair of this laboratory are as follows:

	Antu calculated from nitrogen	*Antu calculated from sulphur*
Sample A	94.44	103.07
Sample B	97.04	100.55

In routine examination of samples of Antu for purity, it would therefore seem preferable to determine total nitrogen rather than total sulphur.

The insolubility of Antu in water renders the application of common reactions of thiourea difficult. Attempts were made to carry out the oxidation method of St. Skramovsky (*Chemical Abstracts*, **38**, 2288) in some other solvent, but without success.

Examinations of prepared Antu rat poisons have been carried out in the following manner:

Weigh an amount of sample equivalent to approximtely 0.2 gm Antu and transfer to a 250 ml Erlenmeyer flask. Add 50 ml acetone and digest or reflux for 15 min. Filter and wash with acetone. Transfer filtrate to a 500 ml Kjeldahl flask. Place on steam bath and evaporate acetone with an air stream. Determine total nitrogen in the residue by Kjeldahl method and calculate to alpha naphthyl thiourea, using the factor: 7.215 times percentage nitrogen equals percentage Antu.

If much greasy material is present, the sample may be digested with a little petroleum ether, filtered on a dry Gooch crucible and washed with petroleum ether. The residue is then heated to 100°C. to remove petroleum ether and the analysis carried out as indicated above beginning "transfer to a 250 ml Erlenmeyer flask."

In case the Antu has been incorporated in material insoluble in acetone, such as starches or sugars, it is advisable first to digest with about 25 m of water, after which 150 to 200 ml of acetone are added and the analysis continued as above beginning "digest or reflux for 15 minutes."

It is recommended* that more specific methods be sought for determination of 1080 and Antu in rodenticides.

REPORT ON DDT

By ELMER E. FLECK (Agricultural Research Administration,
Bureau of Entomology and Plant Quarantine,
Beltsville, Md.), *Associate Referee*

During the past year several new methods and modifications of existing methods for the determination of DDT have been reported. A new colorimetric method for the determination of *p,p'*-DDT has been reported by Bradbury, Higgons, and Stoneman (1). This method is based upon the wine-red color developed by heating *p,p'*-DDT, diethyl sulfate, hydroquinone, and concentrated sulfuric acid. These authors found no interference from *o,p'*-DDT or TDE, but the dehydrochloro derivatives do interfere if present in larger amounts than 5 per cent. The method is sensitive to 0.1 mg of DDT, and the over-all accuracy was 1 per cent. This method is recommended by the authors for use in determining the *p,p'*-DDT content of technical DDT. It has the advantage of being simple and rapid in operation.

Iling and Stephenson (2) have published two variations in the production of color from nitrated DDT. One method provides for the reduction of the nitro groups using hydroxylamine to give an orange color, and the other produces a plum color by the addition of 5 per cent of potassium hydroxide in acetone-methyl alcohol solution. The effect of the compounds usually encountered in DDT other than the *p,p'*-isomer was not discussed.

A new approach to the problem of determining the *p,p'*-DDT content of technical DDT, using solubility methods, has been made by Thorp (3). Small increments of technical DDT were added to a given volume of solvent, and the mixture was then shaken at 25°C. until equilibrium conditions were established. The total amount of DDT added after each increment was then plotted as an abscissa, the amount in solution at each stage of the addition was plotted as an ordinate. As long as all the DDT dissolves, a straight line is obtained. As the solution becomes saturated with any one component, the slope of the line is changed. A similar change in slope occurs as the solution becomes saturated with each component of

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* For report of Subcommittee A and action of the Association, see *This Journal*, 31, 42 (1948).

the technical DDT. When the solution is saturated with respect to all components, a line parallel to the abscissa is obtained. The resulting series of straight lines is projected to the ordinate. From the ratio of the lengths of the segments of the ordinate so obtained it is possible to calculate the percentage of each component present in the DDT. The method can be used to determine TDE as well as *o,p'*-DDT. This method works best in cases where the components are only moderately soluble in the solvent.

LaClair (4) has extended the methods for determining the *p,p'*-DDT content of insecticidal mixtures based on the difference in the rate of dehydrochlorination of *p,p'*-DDT and *o,p'*-DDT. Dehydrochlorination was carried out at 25°C. in order to avoid the dehydrochlorination of *o-p'*-DDT and the hydrolysis of the trichloromethyl group to the corresponding acid.

This is in line with the work of Wain and Martin (5). who showed that refluxing *p,p'*-DDT with 1 *N* alcoholic potassium hydroxide for 30 minutes gave 1.04 mole of chloride ion. They were able to isolate small amounts of 2,2,bis (*p*-chlorophenyl) acetic acid from the reaction mixtures. With 0.1 *N* alkali at 23°C. the quantitative amount of chloride ion was obtained when the reaction was allowed to run for 30 to 60 minutes.

The tentative A.O.A.C. method for the determination of *p,p'*-DDT (6) requires 0.1 *N* alcoholic caustic under reflux conditions for 15 minutes. The results from a collaborative study of this method (7) gave an over-all average of 1.00 mole of chloride ion, but variations on individual runs indicate that some hydrolysis of the trichloromeythyl group may have occurred. It therefore seems advisable to conduct this determination at 25°C.

In last year's report (8) the recommendation was made that the method for the determination of total chlorine in DDT be subjected to further collaborative study, with pure *p,p'*-DDT as the test sample. The results of this study, given in Table 1, show that the method is satisfactory.

W. A. Kirklin and W. W. Haden suggested that the method (1) be modified by use of isopropanol instead of benzene as the diluent. The weighed sample is placed in a 250-ml volumetric flask, 10 ml of benzene is added, and when solution is effected it is made to volume with 99-per cent isopropanol. A 25-ml aliquot is then used for the determination of total chlorine. This modification eliminates the time-consuming step of evaporating the benzene from the aliquot before adding the isopropanol. The results in Table 1 which are indicated by asterisks (*) show this method to be as accurate as the original method. The use of benzene in the extraction of dusts in methods (2) and (3) should be retained to insure complete extraction of the DDT.

The tentative methods for the analysis of insecticidal preparations containing DDT adopted last year (8) did not provide for the analysis of aqueous emulsions. Therefore, methods used in the Bureau of Entomology

TABLE 1.—*Collaborative results on p,p′-DDT and 10 per cent DDT aqueous emulsions*

COLLABORATOR	p,p′-DDT	10 PER CENT DDT EMULSION
	PER CENT CHLORINE	PER CENT DDT FOUND
1	50.56 49.78	9.99 10.04
	Av. 50.17	10.02
2	49.71 49.78	9.96* 9.86*
	Av. 49.75	9.91
3	50.16 49.86	9.92 10.03
	Av. 50.01	9.98
4	50.0 50.1 50.1 50.1	10.18* 10.02* 10.19* 10.20*
	Av. 50.05	10.15
5	49.64 49.47 49.64	9.62 9.36 9.94 9.94
	Av. 49.57	9.71
6	49.95* 49.85* 50.2* 49.9* 50.3* 50.0*	
	Av. 50.05	
General Average	49.93	9.95

* Isopropanol used as solvent.

and Plant Quarantine by R. H. Carter and Anita C. Hazen, and in the Production and Marketing Administration, U. S. Department of Agriculture, by C. G. Donovan, were combined for collaborative testing this year. The combined method originally specified the use of absolute alcohol as the solvent for breaking the emulsion. Later 99-per cent isopropanol was found to have definite advantages in removing water from the sample, as the constant-boiling mixture contains 12.3 per cent by weight of water instead of the 7.7 per cent when ethyl alcohol is used. Since the use of absolute alcohol would require the addition of another reagent to the procedure, the use of isopropanol is recommended. No other change was made in this method.

J. B. LaClair suggests that the last traces of solvent be removed in a current of air after the flask has been cooled to room temperature.

The results of collaborative tests on a 10 per cent DDT emulsion are shown in Table 1. Isopropanol was used in some of the determinations, and no significant difference in the results could be detected.

The following method is to be added to those methods adopted in last year's report:

METHODS FOR DETERMINATION OF TOTAL CHLORINE IN EMULSIONS CONTAINING DDT, SOLVENT, EMULSIFYING AGENT, AND WATER

Weigh a quantity of well-mixed sample containing about 0.75 g of DDT in a tared weighing bottle. Wash into a 100-ml volumetric flask and make to volume with 99-percent isopropanol. Transfer a 10-ml aliquot to a 250–500 ml standard tapered Erlenmeyer flask. Place on a steam bath, and expel the isopropanol and water in a current of air. Remove last traces of solvent and water from the cooled flask. If drops of water still remain, add 10 ml of isopropyanol and repeat the evaporation. Proceed as directed in method (6) *This Journal*, **30**, 65 (1947), line 2, beginning "Add 25 ml of 99% isopropanol. . . . "

The emulsion sent out for analysis was made by mixing equal weights of water and emulsion concentrate. The concentrate contained 20 parts of technical DDT, 21 parts of Deobase (deodorized kerosene), 26 parts of xylene, 26 parts of Velsicol AR-50 (alkylated naphthalenes), and 7 parts of Triton X-100 (an aralkyl polyether alcohol). All parts were by weight.

Grateful appreciation is expressed to the following collaborators who participated in this study:

J. B. LaClair, California Bureau of Chemistry
C. G. Donovan, Production and Marketing Administration, U.S.D.A.
W. A. Kirklin and W. W. Haden, Hercules Powder Co.
R. H. Carter and Anita C. Hazen, Bureau of Entomology and Plant Quarantine, U.S.D.A.

RECOMMENDATIONS*

It is recommended:

(1) That A.O.A.C. method **6.151** for p,p'-DDT be amended as follows: Delete "Reflux alcohol for 15 minutes," and substitute "Place in a water bath at 25°C. for 30 to 60 minutes." Delete " . . . cool to room temperature," and join the remainder of this sentence to the following one.

(2) That method (1), *This Journal*, **30**, 64 (1947), be retained as a tentative method for the determination of total chlorine in DDT, with the following modifications: Delete " . . . and make to volume . . . " thru "Add 25 ml of 99-per cent isopropanol and . . . , " substituting therefor "add 10 ml thiophene-free benzene, dissolve the sample, and then make to

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* For report of Subcommittee A and action of the Association, see *This Journal*, **31**, 42 (1948).

volume with 99-per cent isopropanol. Transfer a 25 ml aliquot to a 250–500 ml standard tapered Erlenmeyer flask. Add . . . ."

That editorial changes be made in methods (2) and (3) to provide for the evaporation of benzene and in (4) to provide for the addition of isopropanol.

(3) That the method given above for the determination of DDT in aqueous emulsions in the absence of other organic chlorine-containing compounds be adopted as tentative.

REFERENCES

(1) BRADBURY, F. R., HIGGONS, D. J., and STONEMAN, J. P., *J. Soc. Chem. Ind.*, **66**, 65 (1947).

(2) ILING, E. T., and STEPHENSON, W. H., *Analyst*, **71**, 310 (1946).

(3) THORP, D., *J. Soc. Chem. Ind.*, **65**, 414 (1947).

(4) LACLAIR, J. B., *Anal. Chem.*, **18**, 763 (1946).

(5) WAIN, R. L., and MARTIN, A. E., *Analyst*, **72**, 1 (1947); *Nature*, **159**, 68 (1947).

(6) *Methods of Analysis, A.O.A.C.*, 1945, 86, sec. **6.151**.

(7) FLECK, E. E., *This Journal*, **28**, 585 (1945).

(8) ——, *Ibid.*, **30**, 319 (1947).

No report was given on fluorine compounds, or on nicotine and nornicotine.

ANNOUNCEMENTS

REFEREE ASSIGNMENTS, CHANGES, AND APPOINTMENTS

James F. Guymon, Agricultural Experiment Station, College of Agriculture, Davis, Calif., Associate Referee on Methyl Alcohol (Miscellaneous Drugs), and on Methanol (Alcoholic Beverages).

William Horwitz, Food and Drug Administration, Minneapolis, Minn., Associate Referee on Phosphatase Test in Dairy Products, in place of George P. Sanders.

Joseph H. Cohen, Chemical Foods Corp., Woburn, Mass., Associate Referee on Gelatine and Gelatine Desserts (Constituents).

R. P. Smith, National Biscuit Company, 449 W. 14th St., New York, N. Y. Associate Referee on Baked Products (Moisture, Ash, Protein, Fat, Crude Fiber, and sugar), to succeed N. H. Walker.

Sidney Kahan, Food and Drug Administration, New York, N. Y., Associate Referee on Glycerol, Vanillin, and Coumarin, in Vanilla and Imitation Vanilla.

Sidney Williams, Food and Drug Administration, Cincinnati, Ohio, Associate Referee on Moisture in Self-rising Flour, and in Pancake, Waffle, and Doughnut Mixes.

CORRECTIONS IN FEBRUARY *Journal*

In the list of Referees, the address of H. C. Heim, given as San Francisco, 2, Calif., should be corrected to School of Pharmacy, University of Colorado, Boulder, Colo.; that of S. Gottleib should be changed to Food and Drug Administration, Washington 25, D. C.

The name of Fred Hillig should be inserted under Dairy Products (Decomposition in Foods), in place of K. L. Harris.

In the method for Indole in Shrimp, Oysters, and Crabmeat, given on page 96, in paragraph on *Color Reagent*, "0.92 ml Phosphoric acid" should read "92 ml."

In par. (6) on page 81, changes in method for soybean flour, next to last line, "see 31.07," etc. the section should be "31.67."

CONTRIBUTED PAPERS

METHOD FOR RAPID DETERMINATION OF TOTAL NITROGEN IN AMMONIUM NITRATE FERTILIZER*

By Ralph D. Miller (Spencer Chemical Co.,
Jayhawk Works, Pittsburg, Kans.)

The method presented here to the Association of Official Agricultural Chemists is Spencer Chemical Company's Standard Procedure N-6 for the determination of total nitrogen in ammonium nitrate fertilizer. This method is specific for ammonium salts and should not be confused with methods for mixed fertilizers. The method mentioned here has been thoroughly tried and tested through the years of war as well as many years previous to the war. The need of such a method has been amply demonstrated by the wide variation of results from different laboratories on the same product. The method described herein is not a new method, but rather a revision of one which has been widely used for many years. The advantage of the method now presented over methods previously available in literature is that it is more reliable for ammonium nitrate solutions and ammonium nitrate fertilizer.

There has been a great deal of work in the past year or so by the late Dr. W. H. Ross[1] of the U. S. Dept. of Agriculture, which was presented in a report on "Nitrogen," by A. L. Prince,[2] New Jersey Agricultural Experiment Station, New Brunswick, New Jersey. In this report, Prince gave conclusive evidence that there is definite need for a more uniform method for determining nitrogen in ammonium nitrate fertilizers. This information is further substantiated by reports received at Spencer Chemical Company from several State laboratories on total nitrogen content of products shipped from this Company.

THE ORIGINAL METHOD

Accurately weigh ca 1 gram sample and transfer to a 250 ml Erlenmeyer flask. Dilute to ca 100 ml with distilled water. Add 25 ml of neutral 20% formaldehyde and heat to 60°C. Cool to 30° and titrate with 0.1 *N* sodium hydroxide using 5 drops of phenolphthalein as indicator. Continue titration until pink remains for 30 seconds.

HISTORY OF THE METHOD

This original method is the standard accepted method for shipments made by contractors to the Army and Navy as covered by the joint Army-Navy Specification 50-11-59F and 51A23, respectively. It was used almost exclusively for this purpose during and since World War II. It is also the accepted method for inspection of ammonium nitrate manufactured by the Ordnance Department in the fertilizer program at present. These specifications and analytical procedures for the government's fertilizer program were established by the Tennessee Valley Authority, and are also used by the T.V.A. in their own plants. The estimated output of the government operated plants in this program amounts to 72,000 tons per month of ammonium nitrate fertilizer. Another significant figure is the 240,000 tons of ammonium nitrate shipped by the Jayhawk Ordnance Works alone during the war years. This material has had duplicate analyses made on each lot by this method, which amounts to about fifty-five thousand analyses. The fact remains that this is only a small part of the actual number of this type of analysis which has been made to date on ammonium nitrate. (When consideration is given to this information, the argument in favor of this basic method's thorough reliability is greatly strengthened.)

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* Presented at The Annual Meeting of the Association of Official Agricultural Chemists held at Washington, D. C. October 20–22, 1947.

[1] Retired.

[2] *This Journal*, 30, 228 (1947).

REVISION OF METHOD

During the operation of the Jayhawk Ordnance Works for the Government, the specifications and methods of analysis for ammonium nitrate were set up and run to conform with U. S. Army Specification PXS-898, dated November 24, 1942 (Picatinny Arsenal). This practice was required at that time because the Ordnance Department was the sole user of the products manufactured at this facility. Since the conversion to private operation in June, 1946, this method has been reviewed as a part of a general program to obtain the maximum degree of efficiency in control methods without loss of accuracy. As is noted in the original procedure, the U. S. Ordnance specification calls for a weighed sample to be treated with formaldehyde (HCHO), heated to 60°C. and cooled to 30°C. before titrating with a standard sodium hydroxide solution. The equation:

$$6HCHO+4NH_4NO_3+4NaOH \longrightarrow C_6H_{12}N_4+4NaNO_3+10H_2O$$

Observation of this method indicated that approximately 33 per cent of the total determination time was consumed in the sample heating and cooling step. Therefore, the following series of tests were made at the laboratories of Spencer Chemical Company on ammonium nitrate over a period of two months and covering seventy-six separate determinations. The comparative samples of nitrate were treated by the original method, except that one pair of samples was heated and cooled, after the addition of formaldehyde and before titration, while the other pair of samples was not heated and was titrated at room temperature.

COMPARISON OF HEATED AND UNHEATED SAMPLES

The results of these analyses are completed in Table 1 of this report. Of the seventy-six determinations made during this work, the average variation was ±0.1 per cent. Thirty-three (33) analyses were lower by the

TABLE 1.—*Variation of "unheated" results from "heated"*

NO.	%NH_4NO_3 UNHEATED	%NH_4NO_3 HEATED	VARIATION IN %	NO.	%NH_4NO_3 UNHEATED	%NH_4NO_3 HEATED	VARIATION IN %
1	83.7	83.8	−0.1	39	82.9	83.0	−0.1
2	83.7	83.6	0.1	40	83.2	83.2	0.0
3	83.7	83.8	−0.1	41	83.9	83.8	0.1
4	83.6	83.7	−0.1	42	83.6	83.6	0.0
5	83.5	83.6	−0.1	43	83.6	83.5	0.1
6	83.4	83.7	−0.3	44	83.4	83.4	0.0
7	83.9	83.7	0.2	45	83.5	83.7	−0.2
8	83.6	83.7	−0.1	46	83.5	83.7	−0.2
9	83.8	83.7	0.1	47	82.9	83.1	−0.2
10	83.5	83.6	−0.1	48	82.8	82.9	−0.1
11	83.3	83.3	0.0	49	83.6	83.3	0.3
12	83.4	83.5	−0.1	50	83.4	83.5	−0.1
13	83.0	83.3	−0.3	51	83.7	83.8	−0.1
14	83.5	83.5	0.0	52	83.6	83.4	0.2
15	83.3	83.1	0.2	53	83.4	83.4	0.0
16	83.2	83.2	0.0	54	83.5	83.5	0.0
17	83.4	83.3	0.1	55	83.8	83.8	0.0
18	83.8	83.8	0.0	56	84.0	83.9	0.1
19	83.8	83.8	0.0	57	83.8	83.7	0.1
20	83.7	83.7	0.0	58	83.6	83.5	0.1
21	83.9	83.9	0.0	59	83.7	83.4	0.3
22	83.5	83.6	−0.1	60	84.0	84.1	−0.1
23	83.6	83.6	0.0	61	83.9	83.9	0.0
24	83.5	83.5	0.0	62	85.0	85.0	0.0
25	83.8	83.8	0.0	63	85.1	84.9	0.2
26	83.8	83.9	−0.1	64	84.2	84.2	0.0
27	83.8	83.7	0.1	65	83.9	84.0	−0.1
28	83.6	83.8	−0.2	66	84.2	84.3	−0.1
29	83.7	83.7	0.0	67	84.0	84.2	−0.2
30	83.6	83.7	−0.1	68	83.7	83.6	0.1
31	83.7	83.6	0.1	69	83.9	83.8	0.1
32	83.5	83.6	−0.1	70	83.3	83.4	−0.1
33	83.5	83.6	−0.1	71	82.7	82.7	0.0
34	83.6	84.0	−0.4	72	82.4	82.7	−0.3
35	83.3	83.7	−0.4	73	82.6	82.2	0.4
36	83.4	83.7	−0.3	74	83.4	83.4	0.0
37	83.6	83.6	0.0	75	83.2	83.4	−0.2
38	83.4	83.4	0.0	76	83.7	83.9	−0.2

No. of Analyses	*Variation per cent*	*% of Total*	*Cumulative % of Total*
24	0.0 (Equal)	31.58	31.58
32	±0.1	42.11	73.69
11	±0.2	14.47	88.16
6	±0.3	7.89	
3	±0.4	3.95	
76		100.00	

unheated method, nineteen (19) were higher, and twenty-four (24) were equal. Of the seventy-six (76) analyses, thirty-two (32) determinations were ±0.1 per cent; eleven (11) determinations were ±0.2 per cent; six (6) were ±0.3 per cent; three (3) were ±0.4 per cent. Table 1 gives the number of analyses in each variation range and the per cent of the total that each represents.

Table 1 shows that 88 per cent of the determinations agree within 0.2 per cent or better, and 74 per cent with 0.1 per cent or better. From the previous experience on nitrate determinations here, it has been found that 0.2 per cent is a reasonable deviation for check samples on the same material. It would appear that the agreement between the two methods is very satisfactory for nitrate determinations since it may be expected to find as much as 1 per cent, or even greater, between two Kjeldahl analyses (using Devarda alloy) on the same material. The method under test was, of course, different from that recommended by the Army. However, in regard to the heating and cooling of these samples nothing could be found in literature giving the formaldehyde reaction with ammonium nitrate where heating and cooling of the sample was specified. On the other hand, the text, "Formaldehyde," by Walker (Reinhold Publishing Company, 1944, page 121), states: "Addition of formaldehyde to ammonium salt solution at room temperature results in the liberation of an acid. When this acid is neutralized or removed, hexamethylenetetramine is obtained. When excess alkali is added to solutions containing ammonium salts, and strong acids plus an excess of formaldehyde, the acid which was originally combined with ammonia may be accurately determined by measuring the amount of alkali consumed. A volumetric method for the determination of NH_4NO_3 is based on the reaction given,

$$6\ HCHO + 4NH_4NO_3 + 4\ NaOH \longrightarrow C_6H_{12}N_4 + 4NaNO_3 + 10\ H_2O.$$

Also in the *Journal of Chemical Education* (November, 1946, Vol. 23, No. 11, page 552), the formaldehyde reaction with NH_3 requires no heating and cooling, and from Allen's Commercial Organic Analysis (5th Ed., Vol. III, page 612), the formal titration does not call for the heating and cooling of the sample, but a titration at room temperature. From these data and from the literature cited, there is believed to be adequate evidence that the analysis of ammonium nitrate can be determined accurately by eliminating the heating and cooling of the sample after the addition of formaldehyde. This change has saved a great deal of time in Spencer laboratories, and with the addition of another slight change, good reproducible results have been obtained.

MODIFICATION OF END POINT

Instead of titration to the first pink color remaining for 30 seconds, the procedure has been changed to continue titration until one drop of .25 normal NaOH produces no perceptible color change at the point of contact. This end point has been found to give better and more consistent results and has been used on all analyses cited in this proposal. It was also used during operation for the Government; therefore, this group has had a great deal of experience with its usage. To use this end point, it is necessary to standardize the sodium hydroxide against C.P. ammonium nitrate with the same end point. It is believed that the *p*H obtained with this end point corresponds to absolute completion of the reaction.

COMPARISON OF STANDARD PROCEDURE N-6 VS. KJELDAHL METHOD

To further test Standard Procedure N-6, the laboratories of Spencer Chemical Company have done a great deal of experimental work in checking this procedure by the various other known analytical procedures. Since the Association of Official Agricultural Chemists at present only recognizes the Kjeldahl method (with various modifications), this was the first to be used in comparing results. It was decided to check various samples from Spencer Chemical Company's graining plants. These samples were taken from shipments and retained in sealed quart fruit jars. The results found in Table 2 are somewhat closer between the two different methods than can be normally expected between two different Kjeldahl tests run on the same sample. This fact was adequately demonstrated in the report by A. L. Prince.[3] Table 1 of his report gave some 31 determina-

TABLE 2.—*Comparison of results by Standard Procedure N-6 and the Kjeldahl method (using Devarda alloy) for determination of total nitrogen in ammonium nitrate fertilizer*

SAMPLE NO.	STD. PRO. N-6	DEVARDA WITH TRAP KJELDAHL
	per cent	*per cent*
1	33.7	33.6
2	33.7	33.7
3	33.6	33.8
4	33.3	33.3
5	33.9	34.0
6	33.4	33.3
7	33.5	33.7
8	33.4	33.3
9	33.5	33.3
10	33.7	33.8
11	33.3	33.1
12	33.1	33.0
13	33.2	33.4
14	33.1	33.2
15	33.2	33.3

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[3] *Loc. cit.*

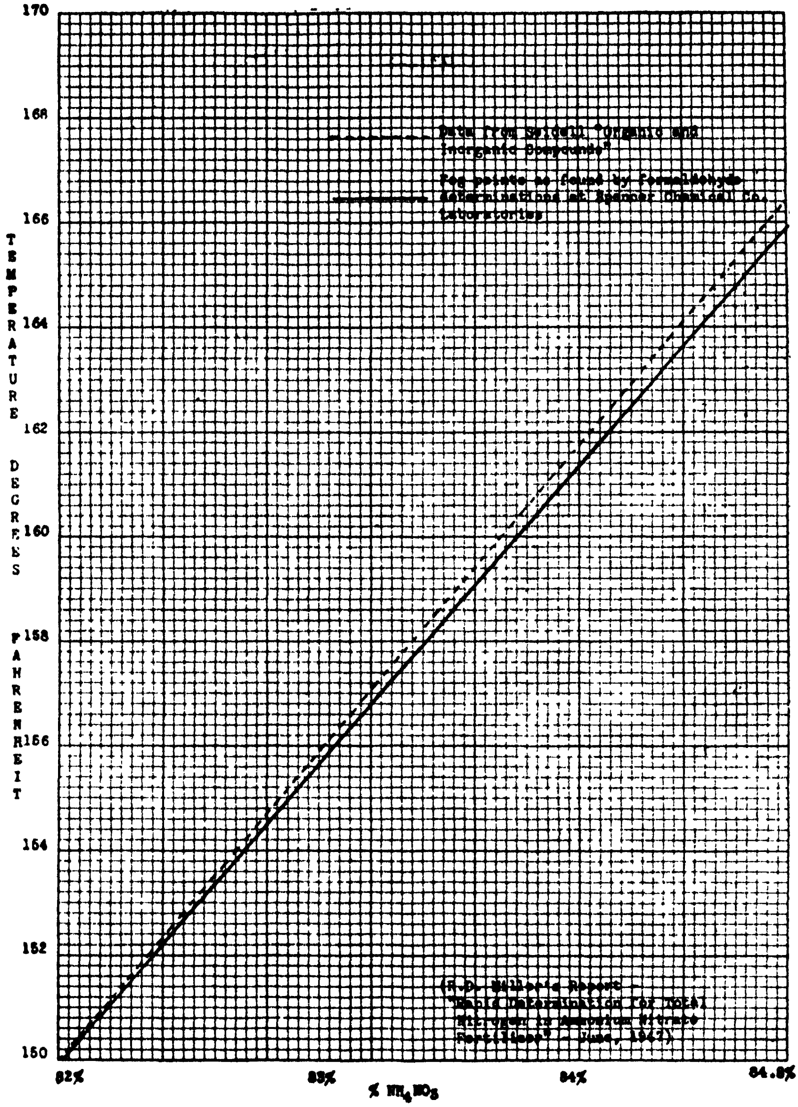
tions on Sample #1 ammonium nitrate with results ranging from 33.92 per cent to 35.18 per cent as found by the collaborators using various modifications of the Kjeldahl method.

COMPARISON OF STANDARD PROCEDURE N-6 VS. FOG POINT METHOD

The next set of comparisons was made using the Fog Point determination which is a standard control method on pure ammonium nitrate solutions made at the Jayhawk Works of Spencer Chemical Company. This determination is based on the freezing point of the concentrated NH_4NO_3 solutions manufactured at this facility. A brief description of this procedure is as follows:

The equipment used consists of a variable speed motor with a glass stirrer; this attached to a solid base to prevent excessive vibration. To the same base a centigrade or fahrenheit thermometer is suspended vertically and fastened rigidly just far enough from the stirrer to prevent breaking. A 150 ml beaker for holding the hot solution is used, and just behind it is placed a light to enable close observance of the transparency of the solution. The hot ammonium nitrate solution is poured into the 150 ml beaker, being careful that no nitrate is crystallized on the sides of the beaker. The amount of solution is about 90 ml. The stirrer is started and the speed controlled to prevent splashing of the solution from the beaker. As the temperature drops and the freezing point is approached, a scum will form on the surface of the warm liquid, which serves to speed the freezing and prevent supersaturation. The "Fog Point" is the point where the clear solution changes to opaque. A temperature reading is taken at this point.

This method was established as a quick control method for operations and has been found to be very accurate and satisfactory for calculations on blending of various solutions and mixes made in the nitrate division. This method was established only after much work had been done in the establishment of its reliability. The complete report on this work is given in a technical report of Spencer Chemical Company No. 904AN-4, by Paul E. Wachter. In order to check the fog point method, a graph was prepared, using information taken from Seidell "Solubilities of Inorganic and Organic Compounds in Water," (page 1043, Vol. II, 2nd Ed.), along with authoritative information published by N. I. Rauich, U.S.S.R. 1933. Our established Fog Point results compare very well with this information, as shown on Graph #1. This Fog Point method has proven superior in every respect for control purposes to the specific gravity determinations used previously. With this confidence in our method, it was felt that the formaldehyde (Standard Procedure N-6) method should be compared with it as a test for reliability. Table 3 shows a comparison of the fog point against the formaldehyde (Standard Procedure N-6)



method on prepared samples, using C.P. ammonium nitrate in distilled water. The samples No. 1, No. 2, and No. 3, were analyzed by the formaldehyde (Standard Procedure N-6) method in three different laboratories by three different analysts. The results are given in percentage ammonium nitrate. It should be noted, on Table 3, the good comparison between the two different methods and the excellent reproducibility of results by the Standard Procedure N-6.

CONCLUSIONS

The Standard Procedure N-6 of the Technical Department of Spencer Chemical Company has been thoroughly tested on ammonium nitrate determinations; it is satisfactory for reproducible results and has the distinct advantage of saving time in the laboratory. It is specific for ammonium salts, and its time requirement is hardly longer than that of determining the strength of nitric acid. The possible argument that mixed acids might have been used during neutralization is eliminated in Standard Procedure N-6 by the colorimetric tests for chlorides and sulfates; these being the salts of the only acids believed capable of entering this type of manufacture.

TABLE 3.—*Correlation of Fog Point analysis along with three separate analyses by different analysts in different laboratories using the formaldehyde method*

(Results are in percentage ammonium nitrate (NH_4NO_3)*

SAMPLE NO.	FOG POINT	NO. 1	NO. 2	NO. 3
1	—	76.64	76.58	76.60
2	77.77	77.93	77.89	78.12
3	77.81	77.57	77.88	78.10
4	79.00	78.98	—	78.86
5	79.39	79.55	79.46	79.14
6	79.52	79.33	79.56	79.39
7	79.96	79.72	79.74	79.72
8	80.14	79.92	79.83	79.92
9	80.66	80.66	80.76	80.85
10	81.18	80.91	80.93	80.77
11	81.71	81.59	81.65	81.94
12	81.88	81.91	81.74	82.04
13	81.88	81.48	81.78	81.88
14	82.23	82.27	82.09	82.03
15	82.72	82.73	82.96	82.95
16	82.84	83.03	83.01	83.00
17	83.19	83.12	83.33	83.27
18	83.49	83.33	83.35	83.63
19	83.63	—	83.78	83.43
20	83.81	84.09	83.79	83.66
21	83.90	83.80	83.91	84.10
22	84.33	84.46	84.34	84.44
23	84.60	84.47	84.42	84.38
24	84.74	84.60	84.39	84.49

* It is to be noted the good comparison between two different methods and the excellent reproducibility over results by the formaldehyde method.

Standard Procedure N-6 is herewith submitted for consideration by the Association of Official Agricultural Chemists as a standard method for determination of total nitrogen in fertilizer grade ammonium nitrate, and of pure ammonium nitrate (solid or in aqueous solutions).

TOTAL NITROGEN IN AMMONIUM NITRATE FERTILIZERS

(Taken from Spencer Chemical Company's Standard Procedure N-6)

REAGENTS

Chemicals Required for Analysis:
20% Formaldehyde Soln.—Prepared from C.P. or Reagent Grade Formaldehyde.
0.25 Normal Sodium Hydroxide Soln.—Standardized against C.P. NH_4NO_3.
Phenolphthalein Indicator.
Distilled Water.
C.P. Ammonium Nitrate.

DETERMINATION

Total Nitrogen:

Place approximately 1.5 gm of sample into a previously weighed 50 ml beaker. The weight of nitrate may be obtained by reweighing the beaker on an analytical balance. The sample thus weighed is dissolved in distilled water and transferred to a 250 ml Erlenmeyer flask. Add 30 ml of 20 per cent formaldehyde (neutral to phenolphthalein) then titrate with 0.25 *N* sodium hydroxide, using phenolphthalein indicator, until the addition of one drop of NaOH produces no perceptible color change at the point of contact.

CALCULATION

$$\frac{\text{Ml NaOH} \times \text{Normality of NaOH} \times 2.802}{\text{Wt. of Sample}} = \%\ \text{Nitrogen}$$

COMPOSITION AND FERTILIZER VALUE OF PHOSPHATE ROCK-MAGNESIUM SILICATE GLASSES[1]

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The production of soluble phosphates by heating mixtures of phosphate rock and siliceous materials, such as blast-furnace slag, calcium and magnesium silicates, magnesium salts and silica, and the like, has been proposed in patents several times during the present century (7, 9, 10, 12, 13). Until recently this type of process attracted little serious attention. Since the publication in 1943 of an article by Walthall and Bridger (11) describing experiments with the production of available phosphate in the form of a glass by fusing phosphate rock with olivine, a growing interest has been aroused in this type of product (2–4, 6, 8). Such a material has recently been put on the market in California under the name *Thermo-Phos*, which is being used both for direct application and in mixed fertilizer (3). The fertilizer possibilities of soluble glasses have recently been emphasized by Badger and Bray (5). This laboratory has investigated samples of experimental and commercial materials produced at various places by fusing phosphate rock with olivine or serpentine with a view towards establishing the physical and chemical composition of these products and determining their nutrient values by means of greenhouse tests. The results of the study are summarized in this report.

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[1] Presented at the Annual Meeting of the Association of Official Agricultural Chemists held at Washington, D. C., October 20–22, 1947.

MATERIALS

Phosphate rock-magnesium silicate glass (Thermo-Phos) now available in commercial quantities is produced by the Permanente Metals Corporation, Permanente, California, by fusing a proportioned mixture of Idaho phosphate rock and serpentine at temperatures approaching 1500°C. in electric-arc furnaces formerly used for ferrosilicon production (3) and quenching the melt in a violent spray of water. The dried granular product is ground to finenesses thought to be appropriate for the market—a −6-mesh material for direct application and a −100-mesh material for use in mixed fertilizers. Samples 2497-a (Table 1) and 2497-b, received in December 1946, are materials of these finenesses, respectively. Apart from fineness the two samples were identical. Sample 2463, received in July 1946 from the same concern, is presumed to be an experimental product. Samples 2497-c and 2533 are regrinds of commercial −6-mesh materials.

TABLE 1.—*Chemical analyses of phosphate-silicate glasses*

SAMPLE NO.	TYPE OF MATERIAL		P_2O_5	CaO	MgO	Al_2O_3	FeO[b]	SiO_2[c]	F
	PHOSPHATE ROCK FUSED WITH—	PRODUCTION							
			%	%	%	%	%	%	%
[a]2497-a	Serpentine	Large-scale	20.04	32.4[d]	17.1[d]	1.8[d]	3.5[d]	23.2[d]	1.70
[a]2463	Serpentine	Experimental	19.75	31.60	18.53	1.95	2.52	22.78	1.65
[a]2488	Olivine	Experimental	22.74	33.76	11.51	2.64	3.82	22.59	2.22
[a]2483	Olivine	Experimental	22.18	31.65	11.98	3.73	3.81	21.66	2.08
2481	Olivine	Experimental	23.61	34.43	11.96	0.59	2.59	22.97	2.21
2489	Olivine	Experimental	19.94	28.33	18.02	2.95	2.38	23.80	1.89
2527	Olivine	Experimental	22.92	35.55	17.82	1.40	1.96	19.24	1.82
—	[e]	Laboratory	13.8	47.0	—	—	—	39.2	—

[a] Material used in greenhouse experiments.
[b] Total Fe.
[c] Without regard for the effect of the presence of fluorine.
[d] Producer's analysis.
[e] A fusion of pure calcium pyrophosphate, calcium carbonate, and cristobalite.

The other numbered samples are experimental products. No. 2481 and 2483 were produced in the State of Washington with the use of Montana phosphate rock and olivine. No. 2489 was prepared some years ago by Walthall and Bridger (11) of the Tennessee Valley Authority. No. 2527 was prepared in Sweden.[1] The laboratory preparation is a homogeneous glass that was prepared several years ago in connection with a phase-rule study. The basic slag (No. 2507) is an imported material that contains 16.9 per cent of total P_2O_5. The alpha phosphate (No. 2496) is the Fused Tricalcium Phosphate produced by the Tennessee Valley Authority and contains 27.68 per cent of total P_2O_5, of which 80.9 per cent was soluble in neutral ammonium citrate and 83.2 per cent in 2 per cent citric acid.

METHODS OF ANALYSIS

Chemical analysis.—The samples (−80- or −100-mesh) were analyzed with the use of standard procedures and appropriate precautions to insure complete solution of the constituent sought. Calcium, magnesium, aluminum, iron and silica were determined in solutions obtained by digesting the sample with hydrofluoric and sulfuric acids and evaporating the digestion until copious fumes of sulfuric acid appeared. Fluorine was determined by distilling the sample with perchloric acid at 125°C. at a rate of 100 ml of distillate per hour and titrating the distillate with thorium nitrate. Since the phosphate glasses do not give up fluorine readily and also vary considerably in this respect, the distillation was prolonged (up to 3 hours) until the titers of successive 50-ml distillates showed that the evolution of fluorine had ceased. Total phosphorus was determined in the solution obtained by treating the sample with aqua regia, evaporating the mixture to dryness to dehydrate the bulky gelatinous silica and dissolving the soluble salts in nitric acid. In order to minimize troublesome caking of the material, the sample was wetted with sufficient water to cover it before the acid was added.

Analyses for citrate-insoluble and citric acid-soluble phosphorus were made in accordance with the official methods for fertilizers (1). Because of the tendency of the material to cake upon the addition of reagents, it was found necessary to shake the flask vigorously immediately after the addition of the sample to the solvent. The citrate-insoluble residue was ignited with magnesium nitrate to destroy the large amount of filter paper and then treated with hydrochloric acid to dehydrate the silica.

Screen analysis.—A sample of 50 grams was used, and the sieves were shaken for 20 minutes on a Ro-Tap machine. The error from dust losses, which ranged from 0 to 1.5 per cent, was usually less than 0.5 per cent and was applied to the finest fraction.

CHEMICAL AND PHYSICAL CHARACTERISTICS OF GLASSES

Chemical composition.—Chemical analyses of eight materials are given in Table 1. Aside from the laboratory preparation of calcium-phosphate-silicate glass, the phosphorus content of the materials ranges from about 19.7 to 23.6 per cent of P_2O_5, and the silica varies more or less independently of the phosphorus from about 19.2 to 23.8 per cent. The greatest variation occurs in the magnesium content (11.5 to 18.5% MgO). The observation that the small amounts of sulfur in the glasses are present as sulfide, indicating reducing conditions during fusion, is the basis for reporting the iron as ferrous oxide. Since the larger part of the fluorine of the phosphate rock remains in the finished product, these furnace materials cannot be viewed as defluorinated phosphates. The presence of fluorine makes fluorapatite a probable crystalline constituent of the product obtained when the furnace charge is allowed to crystallize during slow cooling. Apatite has been identified in the crystallized glass and in poorly quenched products.

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[1] The sample was furnished by P. Gunnar Brundell, Vargon, Sweden.

TABLE 2.—*"Solubility" of phosphate-silicate glasses in neutral ammonium citrate and 2% citric acid solutions*

SAMPLE NO.	TYPE OF MATERIAL		FINENESS	TOTAL P_2O_5	FRACTION OF PHOSPHORUS EXTRACTED		
	PHOSPHATE ROCK FUSED WITH—	PRODUCTION			BY CITRATE	BY CITRIC ACID	DIFFERENCE
			mesh	*per cent*	*per cent*	*per cent*	*per cent*
[a]2497-a	Serpentine	Large-scale	− 6[b]	20.04	62.8	99.0	36.2
[a]2497-b	Serpentine	Large-scale	−100[b]	20.17	83.8	98.3	14.4
2497-c	Serpentine	Large-scale	− 80	20.04	65.3	98.0	32.7
[a]2463	Serpentine	Experimental	− 80	19.75	72.0	99.5	27.5
2533-c	Serpentine	Large-scale	− 25	19.50	76.7	98.9	22.2
[a]2488	Olivine	Experimental	− 80	22.74	84.1	87.0	2.9
[a]2483	Olivine	Experimental	− 80	22.18	78.2	93.0	14.8
2483	Olivine	Experimental	− 10	22.18	32.7	65.3	32.6
2481	Olivine	Experimental	− 80	23.61	67.5	79.0	11.5
2489	Olivine	Experimental	− 80	19.94	84.2	95.6	11.4
2527	Olivine	Experimental	−100	22.92	95.5	98.0	2.5
—	d	Laboratory	− 80	13.83	65.6	93.6	28.0

[a] Material used in greenhouse experiments.

[b] As ground by the producer for the market.

[c] Marketed −6-mesh material reground by the California Bureau of Chemistry for chemical-control analysis. The sample was kindly supplied by F. D. Fowler, Permanente Metals Corporation, Permanente, California.

[d] A fusion of pure calcium pyrophosphate, calcium carbonate and cristobalite.

Phosphorus solubility.—The "solubilities" of several phosphate-silicate glasses in neutral ammonium citrate and 2 per cent citric acid solutions are given in Table 2. Considering first the materials with a fineness of 80 mesh or finer, the citric acid solubilities are with two exceptions (No. 2481 and 2488) well into the nineties, whereas the citrate solubilities range from about 65 to 96. A noteworthy observation (Table 2) has to do with the effect of fineness on the solubility of phosphate rock-magnesium silicate glass. The greater sensitivity of citrate solubility to fineness is further illustrated by the series of results obtained on glass No. 2497-c given in Figure 1, where results obtained on fused alpha phosphate and on im-

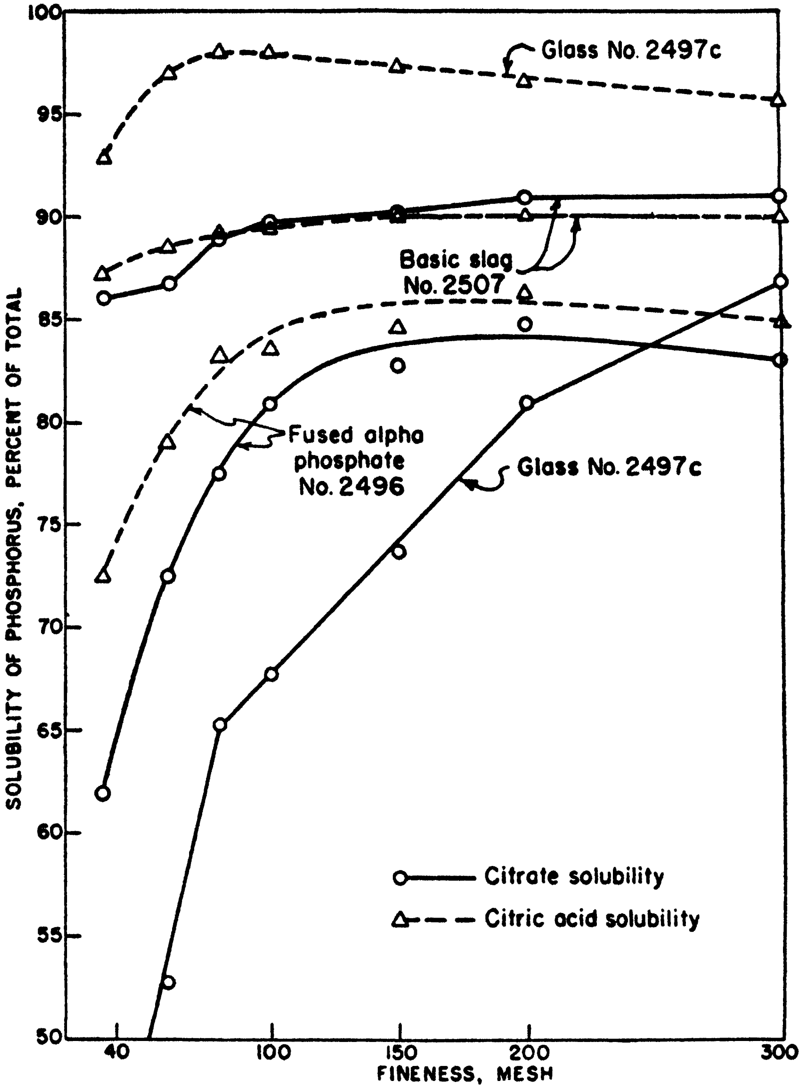

FIG. 1.—Effect of fineness on "solubility" of glass, fused alpha phosphate and basic slag.

ported basic slag are also shown for purposes of comparison. The "solubility" curves for the basic slag lie surprisingly close together. The curves for both the slag and the alpha phosphate fall between the widely separated curves for the phosphate rock-olivine glass. The effect of fineness on the "solubility" of the basic slag is negligible beyond −100 mesh, whereas the citrate solubility of the glass is apparently still increasing markedly at −300 mesh. The decrease in the citric acid solubility of the glass with increase in fineness is attributed to the tendency, already mentioned, of this material to cake under the action of acid.

Screen analyses of the fineness series of phosphate rock-olivine glass included in Figure 1, and of three other similar glasses, are shown in Table 3. Special significance attaches to the two commercially-ground materials with nominal finenesses of −6- and −100-mesh. In these materials the particle-size distribution is such that approximately 80 per cent of the one is coarser than 60 mesh whereas 80 per cent of the other is finer than 300 mesh—a condition that vitiates a comparison of the solubilities of these samples with those of laboratory grinds of similar material. For example, the commercial −100-mesh material showed a citrate solubility of 83.8 per cent (Table 2) in comparison with 67.5 per cent for a −100-mesh sample (Fig. 1) prepared by the authors by grinding the −6-mesh miateral of practically identical chemical composition.

TABLE 3.—*Screen analyses of phosphate rock-magnesium silicate glasses, alpha phosphate, and basic slag*

SAMPLE NO.	MESH SIZE OF SAMPLE	PERCENTAGE OF SAMPLE PASSING SCREEN[b] OF—						
		35 MESH	60 MESH	80 MESH	100 MESH	150 MESH	200 MESH	300 MESH
[a]2497-a	− 6[c]	53.6	17.8	7.8	4.0	1.6	0.4	0.2
[a]2497-b	−100[c]	99.8	99.6	99.4	99.2	97.4	90.4	80.4
2533[d]	− 25	98.3	66.2[e]	—	49.6	38.1	28.3	11.0[f]
[a]2497-c	− 35	100.0	44.4	27.0	19.0	13.0	7.8	5.8
[a]	− 60	—	100.0	76.2	60.2	46.2	33.2	26.4
	− 80	—	—	100.0	85.2	68.6	53.4	44.6
	−100	—	—	—	100.0	91.4	70.2	51.6
[a]	−150	—	—	—	—	100.0	80.0	60.0
	−200	—	—	—	—	—	100.0	83.0
[a]2496	−100	—	—	—	100.0	80.4	50.4	35.8
2507	−100	—	—	—	100.0	93.0	80.8	57.4

[a] Material used in greenhouse experiments.

[b] Screen openings in sieve series were 420, 250, 177, 149, 104, 74 and 46 microns, respectively.

[c] As ground by the producer for the market.

[d] Marketed −6-mesh material reground by the California Bureau of Chemistry for analysis. Sample and screen analysis were kindly supplied by F. D. Fowler, Permanente Metals Corporation, Permanente, California.

[e] A 65-mesh sieve was used.

[f] A 325-mesh sieve was used.

Optical and thermal properties.—The phosphate rock-magnesium silicate fusion products included in this study are essentially all-glass materials. The small amounts of crystalline material often present are mainly quartz, unreacted magnesium silicate and perhaps apatite. To the naked eye the glasses range in color from light green (No. 2527) through brownish grey to black. Observations made on some of the samples under the petrographic microscope[2] are given in Table 4.

All of the iron-bearing materials assume a dirty brown color when they are heated in air for some time below the temperature of complete fusion.

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[2] Examinations were made by J. G. Cady, of this Bureau.

They vary considerably in ease of crystallization in the temperature range of 1000 to 1300°C. For example, No. 2463 annealed at 1200° for 4 hours contained abundant crystals, whereas No. 2483 had not begun to crystallize with 48 hours of heating at this temperature. Determined fusion temperatures lie between 1300 and 1400°C.—1320°, 1335°, 1370° and 1400° (all ±5°) for Nos. 2463, 2497, 2527, and the laboratory preparation, respectively. A few scattered observations indicate that apatite is not always the compound that first crystallizes from the melt, but it is apparently always present in materials that crystallize at temperatures somewhat below the melting point. The low "solubility" of poorly quenched material is, therefore, attributable to apatite formation.

TABLE 4.—*Optical properties of phosphate rock-magnesium silicate glasses*[a]

SAMPLE NO.	INDEX OF REFRACTION	GENERAL APPEARANCE UNDER MICROSCOPE
2497-c	1.62	Smoky greenish-gray glass, some with opaque inclusions; rare irregular masses, almost opaque, showing faint birefringence.
2463	1.62	In general, same as No. 2497-c; rare high-index crystals, presumably pyroxene and olivine; some glass grains contain masses of lath-like crystals.
2483	1.61	Less uniform than No. 2497-c or 2463; gray, light brown, and dark reddish-brown glass containing many black inclusions with a liberal sprinkling of very small (less than 2 microns) high-index crystals.
2489	1.61	Pale brownish-gray to orange glass sprinkled with very small crystals as in No. 2483; rare mineral grains—olivine, sillimanite, quartz, etc.; trace of apatite.
2527	1.61	Clear grayish glass; rare small crystals (quartz, olivine and perhaps some apatite) imbedded in glass.

[a] Examinations were made on −80-mesh materials

Reactivity of glasses in mixtures.—Experiments are in progress to determine the behavior of phosphate rock-magnesium silicate glasses with respect to certain constituents of mixed fertilizers. The results[3] thus far obtained are briefly summarized below.

The reactivity of one glass (No. 2463) with respect to ammonium salts was tested by aspirating air through a suspension of the −80-mesh glass in saturated ammonium nitrate solution at room temperature for a period of 9 days. The glass suspension lost 208 mg. of NH_3 per gram of P_2O_5 in comparison with a loss of 115 mg from a suspension of *alpha* tricalcium phosphate (45.1% P_2O_5) under the same conditions. The behavior of the same glass towards superphosphate was determined by analyzing glass-superphosphate mixtures containing 20, 40, 60 and 80 per cent of superphosphate (20.8% P_2O_5, 1.46% F) at the end of storage periods of 14, 28, 56, and 84 days at 30° and 79.2 per cent relative humidity. All mixtures showed a continuous decrease in water-soluble phosphorus. Nevertheless, no progressive change in citrate-insoluble phosphorus was observed. The insoluble phosphorus at the end of the 14-day period was less than the value calculated from the phosphorus solubility of the initial ingredients by about 1 per cent of P_2O_5, and thereafter showed little change. The mixtures were dry and dusty at the end of the storage period, which indicates lack of moisture, rather than a property of the glass, as the factor that limited reversion of the soluble phosphorus. Mixtures at high moisture contents will, therefore, need to be studied in order to arrive at a definite conclusion as to the compatibility of the glass with superphosphate.

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[3] Data obtained by E. J. Fox, of this Bureau.

GREENHOUSE PROCEDURE

Millet was grown on three soils (−14-mesh) in two-gallon glazed earthenware pots, namely: an Evesboro loamy sand (*p*H 4.8) from Beltsville Maryland, known to be responsive to applied phosphorus, the optimum moisture content of which was found to be 16 per cent of the oven-dry weight of soil; a Nunn,[4] alluvial clay loam soil (*p*H 8.0) from the Experimental Field at Huntley, Montana, which proved to be moderately responsive to applied phosphorus; and Superstition sand (*p*H 8.5), a calcareous soil from Yuma, Arizona, that is known to be highly responsive to applied water-soluble phosphates.

Each pot of Evesboro soil received an application of calcium hydroxide equivalent to 2240 pounds of calcium carbonate per acre, which previous tests had indicated as the lime requirement for an initial *p*H of 6.5. The lime was mixed with all the soil about 3 weeks prior to planting, and during the interim the soil was watered to the optimum moisture content and allowed to air-dry once.

Minor elements were applied in the form of a solution to the Evesboro and Superstition sand soils. The elements applied and their amounts in parts per million parts of soil were: B 1, Cu 0.5, Zn 0.1, Mn 0.1, Fe 0.5, and Mg 10.

All pots were given a basal treatment of nitrogen and potash—nitrogen at the rate of 75 pounds per acre with equal quantities as ammonium sulfate (21.20% *N*) and sodium nitrate (16.48% *N*), and potash in the form of potassium sulfate (54.06% K_2O) at the rate of 150 pounds of K_2O per acre. The phosphorus carriers were applied in the top 3-inch layer of soil by removing the requisite weight of soil from the pot, breaking up the lumps, and then mixing in the fertilizer.

The greenhouse tests, comprising four of the glasses (one at six finenesses), a fused alpha phosphate and double superphosphate at two and three rates of application, were conducted in three experiments (I, II, III), each of which was designed in a manner that would permit statistical treatment of the yield data.

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[4] Tentative classification pending broader study of area in which it occurs.

Experiment I. Twelve phosphate materials were used in this experiment, but only six are pertinent to this discussion. Siberian millet was grown on Evesboro soil. Four of the glasses (one at two finenesses) and double superphosphate were the phosphorus carriers; the rates of application were 50 and 100 pounds of total P_2O_5 per acre; and the design was a randomized block with four replicates. The basal treatment (mixed with all the soil) was applied 22 days prior to seeding, the phosphorus carriers two days before, and the minor elements one day before planting.

The seed (95% germination) was planted March 7, 1947, at the rate of 32 equally-spaced seeds per pot and thinned to 12 plants per pot on March 26. The over-all stand was 90 per cent on the sixth day after planting. The plants, in full head, were harvested on April 11 after a growth period of 31 days.

Experiment II. Golden millet was grown on Evesboro, Nunn, and Superstition sand soils. One glass (two finenesses), a fused alpha phosphate and double superphosphate were the phosphorus carriers, and the rates of application were 50, 100, and 200 pounds of total P_2O_5 per acre. The design (3 replicates) consisted of three randomized split-plot blocks of 39 pots each with all three soils in each block.

The basal treatment and minor elements, both in the form of solutions, were applied to the Evesboro soil and Superstition sand 7 days prior to planting, whereas the basal treatment (solution) without minor elements was applied to the Nunn soil 13 days after seeding. The phosphorus applications were made 8 days prior to seeding.

The millet was planted May 27, 1947, at the rate of 33 equally-spaced seeds per pot and thinned to 10 plants per pot on June 13. Plants were up in all pots in the late afternoon of the second day. The plants, in the boot stage, were harvested on July 8 after a growth period of 42 days.

As early as June 12 the plants growing on Superstition sand showed marked signs of nutrient deficiency—light stripes extending the full length of the leaves were readily apparent. The stripes persisted throughout the growth period and at harvest time were very marked in the plants growing in pots treated with the glass and double superphosphate. On the other hand, pots treated with fused alpha phosphate showed the striping only to a slight extent.

Experiment III. Golden Millet was grown on Superstition sand treated in the manner described for this soil in Experiment II. The dates of planting, thinning, and harvesting were also the same as in the preceding experiment. A glass at four finenesses and double superphosphate was used as phosphorus carriers at 50, 100, and 200 pound rates of application.

The pots (3 replicates) were arranged in a randomized block. Striping of the plant leaves was very pronounced in pots treated with double superphosphate and to a variable extent in pots treated with the glass.

NUTRIENT VALUE OF GLASSES

Statistical analysis of the yields of millet (Tables 5, 6, and 7) show very high significance (Table 8) to variations arising from soil differences, phosphorus carrier, fineness of carrier, and rate of application.

Evesboro Soil.—In greenhouse experiments (I and II) on acid soil the growth response of millet (Tables 5 and 6) to phosphate rock-magnesium silicate glasses ground to -80 mesh or finer was greater than that to double superphosphate in 9 of the 11 instances (Table 9). The coarse material was definitely inferior in both experiments. Statistical significance was attained in all cases at the 100- and 200-pound rates of application. The results show a trend towards lower efficiency of olivine glasses.

TABLE 5.—*Yields of millet on Evesboro soil treated with phosphate rock-magnesium silicate glasses and double superphosphate (Experiment I)*

PHOSPHORUS CARRIER			DRY WEIGHT OF PLANTS IN GRAMS PER POT AT P_2O_5 APPLICATIONS OF—			
			50 LB PER ACRE		100 LB. PER ACRE	
SAMPLE NO.	MATERIAL	MESH SIZE	RANGE	AVERAGE[a]	RANGE	AVERAGE[a]
—	Double superphosphate	− 40	2.55–3.54	3.11[b]	2.76–4.51	3.69
2497-a	Phosphate rock-serpentine glass	− 6	1.08–1.56	1.23	1.19–2.00	1.42
2497-b	Phosphate rock-serpentine glass	−100	2.84–3.90	3.54	5.01–6.51	5.56
2463	Phosphate rock-serpentine glass	− 80	3.23–5.65	4.07	4.69–5.76	5.33
2488	Phosphate rock-olivine glass	− 80	3.00–3.60	3.39	4.40–5.70	5.16
2483	Phosphate rock-olivine glass	− 80	2.35–3.51	3.00	4.32–5.43	4.67

[a] Differences in average dry weights required for significance are 0.80 at 5% level and 1.05 at 1% level.
[b] Dry weights of plants without applied phosphorus were 0.40 to 0.51 with average of 0.44.

Nunn Soil.—The growth responses (Tables 6 and 9), in general, parallel those on Evesboro soil. The 100-mesh glass gave higher responses at the 100- and 200-pound rates of application, whereas the coarse material again was inferior to double superphosphate.

Superstition Sand.—Growth responses of millet (Tables 6 and 7) to the glass are on the average significantly lower than those to double superphosphate (Table 9). Again finely-ground material is superior to coarse material. It would appear from these results that a fineness exceeding 60-mesh is necessary for the glass to compare favorably with double superphosphate. It should be remembered in this connection, however, that the plants growing on this soil showed deficiency symptoms, which lends some uncertainty to the comparisons.

Hartsells Fine Sandy Loam and Fullerton Silt Loam.—In greenhouse experiments reported by Walthall and Bridger (11) the responses of Sudan grass to a phosphate rock-olivine glass on Hartsells fine sandy loam (*p*H

TABLE 6.—*Yields of millet on soils treated with phosphate rock-magnesium silicate glass, fused alpha phosphate, and double superphosphate (Experiment II)*

PHOSPHORUS CARRIER			DRY WEIGHT OF PLANTS[a] IN GRAMS PER POT AT P_2O_5 APPLICATIONS OF—					
SAMPLE NO.	MATERIAL	MESH SIZE	50 LB. PER ACRE		100 LB. PER ACRE		200 LB. PER ACRE	
			RANGE	AVERAGE	RANGE	AVERAGE	RANGE	AVERAGE
			Evesboro soil[b]					
—	Double superphosphate	− 40	14.53–16.97	15.56	17.49–21.55	19.15	15.90–19.41	17.54
2497-a	Phosphate rock-serpentine glass	− 6	10.38–12.92	11.42	7.84–17.93	12.16	12.70–18.00	15.22
2497-b	Phosphate rock-serpentine glass	−100	13.79–18.28	16.20	16.52–19.65	18.44	17.14–19.65	18.51
2496	Fused alpha phosphate	−100	12.95–18.29	15.49	16.23–16.78	16.55	19.51–21.07	20.45
			Nunn soil[c]					
—	Double superphosphate	− 40	16.90–21.62	19.87	18.53–20.97	19.50	18.45–20.62	19.39
2497-a	Phosphate rock-serpentine glass	− 6	17.21–19.85	18.09	15.75–20.44	17.41	16.83–18.30	17.74
2497-b	Phosphate rock-serpentine glass	−100	15.75–18.71	17.64	19.08–23.62	21.52	18.77–21.29	20.15
2496	Fused alpha phosphate	−100	11.48–19.57	15.64	13.19–17.35	14.62	16.67–20.11	18.24
			Superstition sand[d]					
—	Double superphosphate	− 40	4.18– 8.77	5.79	6.37–10.60	8.04	6.88–16.39	11.57
2497-a	Phosphate rock-serpentine glass	− 6	0.07– 1.91	0.88	0.27– 2.18	1.45	1.24– 4.20	2.84
2497-b	Phosphate rock-serpentine glass	−100	4.52–10.13	7.93	5.77–11.00	8.90	3.40–11.85	6.76
2496	Fused alpha phosphate	−100	0.12– 0.84	0.40	0.06– 1.48	0.81	0.15– 1.84	1.02

[a] Difference in average dry weights required for significance are 0.60 at 5% level and 0.78 at 1% level.

[b] Dry weights of plants without applied phosphorus were 1.55 to 4.65 with average of 3.05.

[c] Dry weights of plants without applied phosphorus were 13.84 to 15.44 with average of 14.89.

[d] Dry weights of plants without applied phosphorus were 0.05 to 0.07 with average of 0.06.

TABLE 7.—*Yields of millet on Superstition sand treated with phosphate rock-serpentine glass of different mesh sizes and double superphosphate*[a] *(Experiment III)*

PHOSPHORUS CARRIER			DRY WEIGHT OF PLANTS[a] IN GRAMS PER POT AT P_2O_5 APPLICATIONS OF—					
SAMPLE NO.	MATERIAL	MESH SIZE	50 LB. PER ACRE		100 LB. PER ACRE		200 LB. PER ACRE	
			RANGE	AVERAGE	RANGE	AVERAGE	RANGE	AVERAGE
—	Double superphosphate	− 40	8.01–12.00	9.74	8.52–12.59	11.15	13.32–15.58	14.79
2497-c	Phosphate rock-serpentine glass	− 35	2.57– 3.54	3.07	5.22– 7.26	6.10	7.59–10.51	8.35
		− 60	3.88– 7.70	6.38	4.34–12.17	9.10	9.30–11.09	10.31
		−150	6.62– 8.45	7.74	7.76–10.57	9.42	6.20–10.58	8.91
		−300	10.05–12.76	11.07	8.31–10.42	9.69	6.07– 9.55	8.23

[a] Differences in average dry weights required for significance are 3.21 at 5% level and 4.34 at 1% level.

TABLE 8.—*Analysis of variance*

SOURCE OF VARIATION	DEGREES OF FREEDOM	MEAN SQUARE	DEGREES OF FREEDOM	MEAN SQUARE
	RANDOMIZED BLOCK EXPERIMENTS ON EVESBORO SOIL			
	EXPERIMENT I. 50- AND 100-LB. RATES		FROM EXPT. II. 50- TO 200-LB. RATES	
Total	95	—	63	—
Block	3	2.31†	3	5.11†
Phosphorus carrier[a]	11	11.72‡	3	19.08‡
Rate of application	1	24.95‡	3	18.50‡
Error	80	0.33	54	0.38
	EXPERIMENT II. SPLIT-PLOT BLOCK EXPERIMENT ON EVESBORO, NUNN, AND SUPERSTITION SAND SOILS			
	WHOLE PLOT:		SUB-PLOT:	
Total	8	—	107	—
Soil	2	1955.02‡	—	—
Replicates	2	3.79	—	—
Phosphorus carrier	—	—	3	146.10‡
Rate of Application	—	—	2	37.67‡
Rate × carrier	—	—	6	6.37‡
Rate × soil	—	—	4	3.88‡
Soil × carrier	—	—	6	36.99‡
Soil × carrier × rate	—	—	12	29.28‡
Error	4	21.84	66	0.14
	EXPERIMENT III. FINENESS SERIES ON SUPERSTITION SAND			
Total	44	—		
Block	2	2.74		
Phosphorus carrier	4	40.60‡		
Rate of application	2	25.84†		
Rate × carrier	8	10.00*		
Error	28	3.63		

[a] Of the twelve materials used only six are pertinent to the discussion in this article.
* Significant at 5% level.
† Significant at 1% level.
‡ Significant at 0.1% level.

4.9) and Fullerton silt loam (pH 5.3) were as good as those to double superphosphate.

GLASSES COMPARED WITH FUSED ALPHA PHOSPHATE

Fused alpha phosphate was used as a source of phosphorus for millet growing on Evesboro, Nunn, and Superstition sand soils (Table 6). The growth responses to this phosphorus carrier were in nearly all cases significantly less (Table 9) than those to glasses of comparable fineness.

SOLUBILITY OF PHOSPHORUS COMPARED WITH GROWTH RESPONSE

When the increases in growth response to the glasses in comparison with double superphosphate are set alongside the "solubility" results

TABLE 5.—Increase in growth response of millet on soils treated with phosphate rock-magnesium silicate glasses and fused alpha phosphate in comparison with double superphosphate

PHOSPHORUS CARRIER				INCREASE[a] IN AVERAGE DRY WEIGHT IN GRAMS PER POT AT P_2O_5 APPLICATIONS OF—		
SAMPLE NO.	MATERIAL	MESH SIZE	EXPERIMENT NO.	50 LB. PER ACRE	100 LB. PER ACRE	200 LB. PER ACRE
			Evesboro soil			
2497-a	Phosphate rock-serpentine glass	− 6	I	−1.88†	−2.27†	—
	Phosphate rock-serpentine glass		II	−4.14†	−6.99†	−2.32†
2497-b	Phosphate rock-serpentine glass	−100	I	+0.43	+1.87†	—
	Phosphate rock-serpentine glass		II	+0.64*	−0.71*	+0.97†
2463	Phosphate rock-serpentine glass	− 80	I	+0.96*	+1.64†	—
2488	Phosphate rock-olivine glass	− 80	I	+0.28	+1.47†	—
2483	Phosphate rock-olivine glass	− 80	I	−0.11	+0.98*	—
2496	Fused alpha phosphate	−100	II	−0.07	−2.60†	+2.91†
			Nunn soil			
2497-a	Phosphate rock-serpentine glass	− 6	II	−1.78†	−2.09†	−1.65†
2497-b	Phosphate rock-serpentine glass	−100	II	−2.23†	+2.02†	+0.76*
2496	Fused alpha phosphate	−100	II	−4.23†	−4.88†	−1.15†
			Superstition sand			
2497-a	Phosphate rock-serpentine glass	− 6	II	−4.91†	−6.59†	−8.73†
2497-b	Phosphate rock-serpentine glass	−100	II	+2.14†	+0.86†	−4.81†
2497-c	Phosphate rock-serpentine glass	− 35	III	−6.67†	−5.05†	−6.44†
	Phosphate rock-serpentine glass	− 60	III	−3.36*	−2.05	−4.48†
2497-c	Phosphate rock-serpentine glass	−150	III	−2.00	−1.73	−5.88†
	Phosphate rock-serpentine glass	−300	III	+1.33	−1.46	−6.56†
2496	Fused alpha phosphate	−100	II	−5.39†	−7.23†	−10.55†

[a] Plus sign indicates a yield greater than that with double superphosphate.
* Significant at 5% level
† Significant at 1% level.

TABLE 10.—*Availability of Phosphate Rock-magnesium Silicate Glasses by "Solubility" and Growth-response Methods*

DESCRIPTION OF GLASS				SOLUBILITY[a] OF PHOSPHORUS IN—		INCREASE IN GROWTH RESPONSE[b] IN GRAMS PER POT AT RATE OF APPLICATION OF—			SOIL USED
NO.	MADE WITH	PRODUCTION	MESH SIZE	NEUTRAL AMMONIUM CITRATE	2 PER CENT CITRIC ACID	50 LB. PER ACRE	100 LB. PER ACRE	200 LB. PER ACRE	
2497-a	Serpentine	Large-scale	− 6	63	99	−1.88†	−2.27†	—	Evesboro
						−4.14†	−6.99†	−2.32†	Evesboro
						−1.78†	−2.09†	−1.65†	Nunn
						−4.91†	−6.59†	−8.73†	Superstition
2497-b	Serpentine	Large-scale	−100	84	98	+0.43	+1.87†	—	Evesboro
						+0.64	+0.71*	+0.97†	Evesboro
						−2.23†	+2.02†	+0.76*	Nunn
						+2.14†	+0.86†	−4.81†	Superstition
2497-c	Serpentine	Large-scale	− 35	43	93	−6.67†	−5.05†	−6.44†	Superstition
			− 60	53	97	−3.36*	−2.05	−4.48†	Superstition
			−150	74	97	−2.00	−1.73	−5.88†	Superstition
			−300	87	96	+1.33	−1.46	−6.56†	Superstition
2463	Serpentine	Experimental	− 80	72	100	+0.96*	+1.64	—	Evesboro
2488	Olivine	Experimental	− 80	84	87	+0.28	+1.47	—	Evesboro
2483	Olivine	Experimental	− 80	78	93	−0.11	+0.98	—	Evesboro

[a] In percentage of the total phosphorus.
[b] In comparison with that to double superphosphate at rate of 100 pounds of P_2O_5 per acre (Table 9).
* Significant at 5% level.
† Significant at 1% level.

(Table 10), one noteworthy comparison stands in bold relief. The difference between the fineness of the −6- and −100-mesh materials, which is reflected markedly in growth response, is indicated to a moderate extent by citrate solubility and is not shown at all by citric acid solubility. The trend of the results for the fineness series applied to Superstition sand at 50- and 100-pound rates indicate similar relationships. It would appear therefore that, as concerns immediate utilization of the phosphorus by plants, citric acid solubility, and perhaps also citrate solubility, overrates the availability of coarse glass.

It has been suggested that in the case of the coarse material, which probably consists of granules as they come from the quenching operation, the nature of the surface rather than its extent may be the factor that limited the utilization of this material by growing plants. Actually the −6-mesh material does consist of rods and spherical and vesicular granules, many with attached filament, with mirror-like surfaces, all of which indicates a quenched surface little disturbed by grinding. The consideration is, then, a quenched surface in comparison with a surface of fracture. The data at hand do not permit a decision on this question.

SUMMARY

Seven commercial phosphate rock-magnesium silicate glasses, including one sample of large-scale production and experimental glasses produced with the use of both serpentine and olivine, were analyzed for P_2O_5, CaO, MgO, Al_2O_3, FeO, and F, and their solubilities in neutral ammonium citrate and 2 per cent citric acid were determined.

Selected glasses were subjected to screen analysis, to a study of fineness-solubility relationships, to petrographic examination, to crystallization tests, to reactivity tests in mixtures, and to plant-response tests on Evesboro, Nunn, and Superstition sand soils in the greenhouse, with the use of fused alpha phosphate and double superphosphate as comparison materials.

The major constituents of the glasses are P_2O_5 (19.75–23.61%), SiO_2 (19.24–23.80%), CaO (28.33–35.55%), and MgO (11.51–18.53%); other important constituents are Al_2O_3 (0.59–3.73%), FeO (1.96–3.82%) and F (1.65–2.22%).

The effect of fineness on citrate solubility is much more pronounced in the case of these glasses than in the cases of basic slag and fused alpha phosphate. The citrate solubilities of a glass at finenesses of −60 and −300 mesh, respectively, were 52.8 and 86.8 per cent in comparison with 86.7 and 91.0 for a basic slag at the same finenesses. Citric acid solubility did not change markedly over this range of fineness.

The glasses vary considerably in rate of crystallization when they are annealed at temperatures below the melting point (1300 to 1400°C.). Apatite is always an important constituent of crystallized samples, and

the lowered phosphorus solubility accompanying crystallization is attributable to apatite formation.

The glass caused a marked loss of ammonia when it was suspended in an aqueous solution of ammonium nitrate. Glass-superphosphate mixtures (20 to 80% superphosphate) in indecisive tests showed continuous decreases in water-soluble phosphorus without progressive changes in citrate-insoluble phosphorus.

In greenhouse tests on Evesboro (pH$=4.8$) and Nunn (pH$=8.0$) soils, the growth response of millet to the glasses (-80-mesh or finer) was greater than that to double superphosphate in twelve of the twenty cases, whereas on Superstition sand (pH$=8.5$) the response was usually less than that to double superphosphate. Coarse glass was inferior to finely-ground glass on all three soils, and a fineness exceeding 60 mesh is indicated as necessary for favorable comparison of the glass with double superphosphate.

LITERATURE CITED

(1) *Methods of Analysis, A.O.A.C.* (1945).
(2) Anonymous, *Chem. Met. Eng.*, **53**, 194, 196 (July 1946).
(3) ——, *Chem. Eng.*, **53**, 212, 214 (Nov. 1946).
(4) ——, *Ibid.*, **54**, 214–15 (May 1947).
(5) Badger, A. E., and Bray, R. H., *Chem. Met. Eng.*, **52**, 112–13 (1945).
(6) Callaham, J. R., *Chem. Eng.*, **53**, 116, 118 (Oct. 1946).
(7) Heskett, J. A., Brit. Patent 435,763 (Sept. 23, 1935).
(8) Moulton, R. W., Trans. Am. Inst. Chem. Eng., in *Chem. Eng. Progress*, **43**, 163–64 (1947).
(9) *Rhenania Verein Chemische Fabriken*, Austrian Patent 105,806 (March 10, (1927).
(10) ——, Swiss Patent 124,686 (Feb. 16, 1928).
(11) Walthall, J. H., and Bridger, G. L., *Ind. Eng. Chem.*, **35**, 774–77 (1943).
(12) Wiborgh, J. G., Swedish Patent 18,401 (Jan. 16, 1903).
(13) Wolters, W., U. S. Patent 721,489 (Feb. 24, 1903).

EFFECT OF ALCOHOL CONCENTRATION AND SATURATION OF THE ACID ALCOHOL WITH K_2PtCl_6 IN THE ANALYSIS OF POTASH IN FERTILIZERS*

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In the official A.O.A.C. method for determining potash in fertilizers the K_2PtCl_6 is treated with acid alcohol and washed with 80% alcohol (1). Ford and Hughes (2) showed that an increase in the concentration of the alcohol and acid alcohol resulted in an increase in the amount of potash obtained in the determination of potash in fertilizers. Mitchell and Ford (3) showed that in the case of potash determined from pure potassium chloride the increases obtained with the higher alcohol concentrations were due to true potash. Ewan, Ford, and Schall (4) showed that the increases in potash obtained in analyzing mixed fertilizers when 95% alcohol was used in place of 80% alcohol were also true potash. The same workers also reported a large increase of potash when the 80% acid alcohol was saturated with K_2PtCl_6 and a smaller increase when the 95% acid alcohol was saturated with K_2PtCl_6. In each case, however, the increases were found to be true potash.

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TABLE 1.—*Effect of concentration of ethanol and saturation of acid alcohol with K_2PtCl_6 on K_2O values*

SAMPLE	85% ACID ALCOHOL +ALCOHOL			90% ACID ALCOHOL +ALCOHOL		
	% K_2O			% K_2O		
	High	*Low*	*Av.*	*High*	*Low*	*Av.*
A. (0-12-12)						
Unsat.	13.09	13.00	13.05*	13.19	13.12	13.16*
Sat.	13.36	13.25	13.33	13.31	13.26	13.29
B. (0-20-20)						
Unsat.	21.18	21.11	21.15	21.30	21.20	21.26
Sat.	21.70	21.51	21.58	21.42	21.40	21.41
C. (0-9-27)						
Unsat.	28.20	27.88	28.00	27.88	27.88	27.88
Sat.	28.24	28.16	28.20	28.20	28.13	28.15
D. (8-8-8)						
Unsat.	8.12	8.09	8.11	8.37	8.19	8.30
Sat.	8.56	8.45	8.50	8.42	8.34	8.37
E. (4-12-8)						
Unsat.	8.60	8.48	8.54	8.58	8.45	8.52
Sat.	9.05	8.68	8.86	8.70	8.59	8.65

* Average in each case is the result of 3 analyses

The purpose of this work was to supplement that of Ewan, Ford, and Schall (4) by determining the influence of 85 and 90% ethanol with and without prior saturation with K_2PtCl_6 upon the potash values and the purity of the precipitate obtained.

To test the purity of the K_2PtCl_6 obtained, the weighed salt in each case was reduced for platinum recovery and estimation of the chloride by a Volhard titration. To facilitate the reduction the salt in each case was dissolved in hot water, transferred to a new, unscratched 250 ml. beaker, and if necessary concentrated to a volume of about 75 ml. While boiling, 2–5 ml. of 40% formic acid was added and as soon as reduction started a piece of filter paper on a glass rod was used to prevent sticking. As soon as the reduction was complete, the reduced platinum black together with the filter paper was filtered on a tared asbestos-padded Gooch, dried at 100°C., ashed in a muffle at 600°C. for about 5 hours, cooled, and weighed. The filtrate from the reduction in each case was collected, made to volume and aliquots taken for the estimation of the chloride by the Volhard titration method. The endpoint of the titration was sharpened by coagulating the precipitated silver chloride by shaking it with 1–2 ml. of nitrobenzene and filtering it off before the final titration was made.

RESULTS

The results of this work (Table 1) confirm and extend those of Ewan, Ford, and Schall (4) in showing that the increases in potash are smaller with the higher concentrations of alcohol. On the other hand, saturation of the 85% acid alcohol with K_2PtCl_6 gave larger increases than saturation of 90% acid alcohol.

TABLE 2.—*Effect of ethanol concentration and saturation of the acid alcohol on the purity of the K_2PtCl_6*

SAMPLE	PLATINUM (GRAMS)			CHLORIDE (GRAMS)		
	THEORETICAL	FOUND	DIFFERENCE	THEORETICAL	FOUND	DIFFERENCE
			85% Unsaturated			
A.	.0676	.0674*	-.0002	.0737	.0737*	.0000
B.	.1097	.1093	-.0004	.1195	.1193	-.0002
C.	.1453	.1453	.0000	.1582	.1581	-.0001
D.	.0420	.0417	-.0003	.0458	.0465	+.0007
E.	.0441	.0438	-.0003	.0483	.0485	+.0002
			85% Saturated			
A.	.0690	.0687	-.0003	.0753	.0748	-.0005
B.	.1117	.1115	-.0002	.1220	.1207	-.0013
C.	.1461	.1460	-.0001	.1582	.1579	-.0003
D.	.0447	.0452	+.0005	.0491	.0488	-.0003
E.	.0459	.0458	-.0001	.0501	.0500	-.0001
			90% Unsaturated			
A.	.0681	.0680	-.0001	.0743	.0746	+.0003
B.	.1101	.1098	-.0003	.1201	.1201	.0000
C.	.1444	.1441	-.0003	.1575	.1557	-.0018
D.	.0430	.0428	-.0002	.0469	.0473	+.0004
E.	.0442	.0449	+.0007	.0482	.0483	+.0001
			90% Saturated			
A.	.0689	.0688	-.0001	.0751	.0744	-.0007
B.	.1109	.1110	+.0001	.1210	.1204	-.0006
C.	.1459	.1459	.0000	.1590	.1572	-.0018
D.	.0434	.0432	-.0002	.0472	.0472	.0000
E.	.0448	.0442	-.0006	.0488	.0489	+.0001

* Each value is the average of 3 analyses.

The analysis of the K_2PtCl_6 residues for platinum and chloride indicate that within the limits of experimental error the potash obtained is true potash whether obtained by an increase in alcohol concentration or by saturation of the corresponding acid alcohol with K_2PtCl_6 (Table 2).

LITERATURE CITED

(1) Official and Tentative Methods of Analysis, A.O.A.C., 6th Ed., pp. 31–32 (1945).
(2) Ford, O. W., and Hughes, C. W., *Ind. Eng. Chem., Anal. Ed.*, 14, 217–18 (1942).
(3) Mitchell, H. L., and Ford, O. W., *Ind. Eng. Chem., Anal. Ed.*, 15, 56 (1943).
(4) Ewan, M. A., Ford, O. W., and Schall, E. D., (at press) (Journal paper #270) of the Purdue Agr. Exp. Sta.

A PROCEDURE FOR THE DETERMINATION OF THE PHOSPHORUS CONTENT OF HEXAETHYL TETRAPHOSPHATE AND OF CERTAIN OTHER ORGANIC PHOSPHATES*

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Hexaethyl tetraphosphate, $C_{12}H_{30}O_{13}P_4$, has been reported to be an ideal contact insecticide for the control of aphids and spider mites. "Known in Germany under the trade name *Bladan*, the compound is used as a contact insecticide in either liquid or dust formulations. It has been found effective as a spray in liquid dilutions ranging from 1:1000 to 1:4000 and in concentrations of from 3 to 5 per cent when incorporated with dust carriers."[1]

Several questions arose when this organic phosphate was suggested as an ideal means of insect control in the greenhouse in which plant cultures were being used to establish the fertilizer effectiveness of certain new types of phosphorus compounds—Would the spray residues register to enhance the true phosphorus content of the crops; would the contamination affect plant response through a direct intake of phosphorus into the above-ground growth, or through an uptake from the soil? An additional consideration was whether substantial inputs of the organic phosphate would persist in un-ionized form in the soil and thus provide an effective phosphorus carrier resistant to fixation. Therefore, it was necessary to determine the phosphorus content of the hexaethyl tetraphosphate and its hydrolytic behavior under laboratory treatments and after incorporation into the soil. In the initial analysis of the first sample of that compound, the digestions were those imposed in the determination of the phosphorus content of metaphosphate fertilizers, since those digestions had been found adequate to bring into ortho form all of the other inorganic phosphates used in earlier experiments.[2] In exploratory pot culture trials of the fertilizer effectiveness of the first sample of hexaethyl tetraphosphate, the soil incorporations were based upon the P_2O_5 equivalences indicated by the initial analysis. Upon that basis, crop response to the hexaethyl tetraphosphate exceeded the response to the inorganic phosphates, when phosphorus input was 80 pounds of P_2O_5 per acre surface, half-depth incorporation. This apparent superiority was nullified, however, by the revelation that organically integrated phosphorus was not converted to PO_4 by the conventional prefatory acid digestion of aliquots. Consequently, the organic phosphate incorporations supplied the soil with quantities of phosphorus far greater than those intended, and greater than those supplied by the inorganic ortho phosphate controls.

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* Based upon findings obtained in a study conducted in the Cooperative Chemical Research Laboratory of the Department of Chemical Engineering, Tennessee Valley Authority, at the Agricultural Experiment Station of The University of Tennessee.

† Presented at the Annual Meeting of the Association of Official Agricultural Chemists held at Washington, D. C., October 20–22, 1947.

[1] *Ind. Eng. Chem., News Edition*, 25, 1069, April 1947.

ANALYTICAL STUDIES

Preliminary observations indicated that the analysis of the liquid hexaethyl tetraphosphate called for both oxidation and ionization, prefatory to the molybdate precipitation. The dual objective was sought through a nitric acid + perchloric acid digestion of a 10-ml. aliquot of a 250-ml. solution of a 1-gm. charge of the organic phosphate. This aliquot had a P_2O_5 equivalence of approximately 20 mgm. Extended to 5 hours, the combined acid digestion gave a phosphorus recovery of only 40 per cent, which was about two-thirds of the recovery obtained through a comparable digestion with aqua regia. Further analytical studies established the fact that the assumed requirement for oxidative treatment was not essential. Comparisons demonstrated that complete transition of the phosphorus content of the organic phosphate into ionized form could be effected by prolonged digestion in relatively concentrated hydrochloric acid, in concentrated nitric acid and in aqua regia. The analytical results shown in Table 1 were obtained when the first sample of hexaethyl tetraphosphate and the two other samples of that compound were subjected to the described analytical treatments and durations.

Ionization had not occurred in the aqueous medium after the solution had stood quiescent 48 hours at room temperature, and but little ionization was induced when a 1-gm. charge of the sample S-1275 was diluted with 150 ml. of water and boiled 3 hours, down to 10 ml. After 24 hours of standing in the aqua regia system at room temperature, a mere trace of phosphorus transition to ortho form had occurred. Moreover, *only small fractions of the potential transitions were induced by conventional digestions in either HNO_3 or HCl at 120°C., even when the digestions were for periods 8 to 10 times longer than those ordinarily imposed.* The quantity of generated PO_4 showed a progressive increase when 10-ml. aliquots of the organic phosphate were digested in aqua regia at 120°C. up to 24 hours. The transitions then registered were apparently absolute, since they were virtually identical with those registered by 20-, 24-, and 28-hour digestions. Upon basis of those determinations, sample S-1275 was a 98.75 per cent product in relation to formula.

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[2] *Ind. Eng. Chem.*, 29, 224 (1937).

Since the sample of hexaethyl tetraphosphate yielded a distinct odor, it seemed desirable to ascertain whether the observed volatility would entail a loss of phosphorus. Therefore, a 150-ml. aqueous solution of 1 gram of the organic phosphate S-1275 was evaporated to 10 ml. through two hours of gentle boiling in a beaker. The resultant concentrate then was dilute to volume in a 250-ml. flask and 10-ml. aliquots were subjected to digestions identical with those imposed upon corresponding aliquots of an untreated aqueous solution of the initial sample. Since the analytical finding of 55.2 per cent P_2O_5-equivalence for the boiled aliquot was identical to the value shown for the aqueous aliquot that was acid-digested, as in Table 1, it is obvious that no loss of phosphorus occurred during the prolonged unrefluxed boiling.

TABLE 1.—*Effect of various ionization treatments upon the conversion of the phosphorus content of three commercial hexaethyl tetraphosphates into ortho form*

TREATMENT OF ALIQUOT		P_2O_5 EQUIVALENCE OF PRODUCT[a]		
REAGENT	DIGESTION PERIOD	S-1271[b,c]	S-1275[b,c]	S-1280[d,e]
In water at room temperature	48 hrs.	None	None	—
In aqua regia at room temperature	24 hrs.	Trace	Trace	Trace
In acids		—	2.1	—
5 ml. HNO_3, 120° C.[f]	15 min.	6.2	—	—
5 ml. HNO_3, boiling	30 min.	—	9.0	—
5 ml. HNO_3, 120° C.[f]	20 hrs.	—	46.1	—
5 ml. HCl, 120° C.[f]	30 min.	4.4	—	—
10 ml. HCl, boiling	30 min.	4.7	19.0	—
10 ml. HCl, 120° C.[f]	1 hr.	6.1	—	—
5 ml. HCl, boiling	30 min.	9.2	—	—
5 ml. HCl, 120° C.[f]	2 hrs.	8.7	—	—
5 ml. HCl, 120° C.[f]	20 hrs.	8.8	55.2	26.9

[a] Determined volumetrically on a 10 ml treated aliquot of a solution of the sample, 1-gm. charge diluted to 250 ml.

[b] Aqueous solution.

[c] Theoretical P_2O_5 content 56%, upon basis of formula $C_{12}H_{30}O_{13}P_4$ for hexaethyl tetraphosphate.

[d] Theoretical P_2O_5 content 28%, upon basis of formula $C_{12}H_{30}O_{13}P_4$ for hexaethyl tetraphosphate (product labelled as 50% solution. The inert ingredient was an organic solvent).

[e] Alcoholic solution of sample.

[f] Slightly below boiling point of the mixture of sample and acid

When a 10-ml. aliquot of the aqueous solution of hexaethyl tetraphosphate was evaporated to dryness, the result was a black charry mass, which contained a white substance that was insoluble in dilute nitric acid. Aliquots of the 250-ml. solution of a 1-gm. charge of that phosphate also were subjected to treatments with the peroxides of hydrogen, calcium, sodium, and barium, prefatory to the presently prescribed acid digestion, but none of these effected the desired transition of the organic phosphorus into ortho form. The period of digestion requisite for the complete transition of sample S-1275 was diminished by several hours when 10-ml. aliquots were processed by evaporation with a like volume of a saturated solution of magnesium nitrate, cautious incineration, and dissolution of the incinerate in dilute nitric acid. Although such processing served to effect complete transitions of the phosphorus content of 10-ml. aliquots of certain organic phosphates, it did not induce complete transitions in some cases. Moreover, such processing of aliquots introduces undesirable mechanical features, and is not recommended presently. The prescribed "*Analytical Technique*" is the only direct digestion procedure yet found to meet fully the requirement for the determination of the P_2O_5-equivalences of those samples of hexaethyl tetraphosphate that have been subjected to the present analytical evaluations.

ANALYTICAL TECHNIQUE FOLLOWED IN THE DETERMINATION OF THE PHOSPHORUS CONTENT OF HEXAETHYL TETRAPHOSPHATE

The following steps proved requisite for the conversion of the highly stable hexaethyl tetraphosphate to the form essential for full phosphorus recovery through precipitation as ammonium phosphomolybdate:

A 1-gm. charge of the sirupy organic phosphate was weighed into a 250-ml. flask, diluted to volume and mixed. A 10-ml. aliquot was transferred to a 200-ml. Erlenmeyer flask fitted with tube for air refluxing. Additions of 5 ml. each of concentrated hydrochloric and nitric acids were made and the solution was digested at least 16 hours at 120°C. The digestate was allowed to cool and the sides of the flask and the reflux tube were washed with a small volume of water. The diluted digestate was boiled in an open flask to expel free chlorine. The solution was allowed to cool, made slightly alkaline by means of ammonium hydroxide (1+1), and again allowed to cool. A few drops of methyl orange were added and the system was made slightly acidic through addition of nitric acid (1+1). Phosphorus content was determined as in the A.O.A.C. volumetric procedure for fertilizers.[3]

OBSERVATIONS CONCERNING OTHER ORGANIC PHOSPHATES

Since hexaethyl tetraphosphate and similar organics may be used extensively, there should be an analytical procedure adapted to the verification of the phosphorus content indicated by the formula. The experience with the hexaethyl tetraphosphate was applied in the analytical evaluation of certain other organic phosphates, several of which were subjected to the conditions detailed in Table 2. The P_2O_5 values obtained by the procedure prescribed for a 24-hour digestion of the hexaethyl tetraphosphate were in accord with those computed from the formulas attributed to three of the samples, whereas only partial P_2O_5 values were registered in the case of samples S-1277 and S-1278 purported to be tricresyl phosphate and triphenyl phosphate, respectively.

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[3] *A.O.A.C. Official and Tentative Methods of Analysis*, Sixth Edition, 1945.

TABLE 2.—*The progressive conversion of the phosphorus of certain organic phosphates into ionized ortho form by means of Aqua Regia digestion*[a]

DIGESTION PERIOD	DETERMINED P_2O_5 VALUES[b]—PERCENTAGES						
	S-1271[c,d]	S-1275[c,d]	S-1280[e,f]	S-1276[c,g]	S-1277[f,h]	S-1278[f,i]	S-1279[c,j]
Hrs.							
¼	7.0	3.9	—	—	—	—	[k]
½	8.6	5.0	—	—	—	—	—
1	11.4	7.4	None	None	None	None	17.10
2	15.6	11.4	—	Trace	None	None	—
3	25.4	17.1	—	2.8	None	None	—
4	27.6	22.2	None	3.2	None	None	36.0
5	35.6	30.6	—	9.4	—	—	—
6	40.1	38.6	—	—	—	—	—
7	47.6	44.6	—	—	—	—	—
16	53.7	54.8	23.7	—	3.0	11.9	57.2
20	57.0	54.8	—	37.4	—	—	—
24	57.5	55.1	28.7	37.3	4.9	18.1	57.5
28	—	55.3	—	—	—	—	—

[a] 10 ml. aliquots of 1 gm. to 250 ml. of sample solution digested with 10 ml. aqua regia in flask with air reflux at 120° C., the temperature just below boiling point.

[b] Determined volumetrically after neutralization of digested aliquot.

[c] Aqueous solution.

[d] Theoretical P_2O_5 content 56%, upon basis of formula $C_{12}H_{30}O_{13}P_4$ for hexaethyl tetraphosphate.

[e] Same as (d), but labelled as containing "50% active ingredients."

[f] Alcoholic solution.

[g] Theoretical P_2O_5 content 38.6%, upon basis of formula $(C_2H_5)_3O_3PO$ for triethyl phosphate.

[h] Theoretical P_2O_5 content 19.2%, upon basis of formula $(C_6H_4CH_3)_3O_3PO$ for tricresyl phosphate.

[i] Theoretical P_2O_5 content 21.75%, upon basis of formula $(C_6H_5)_3O_3PO$ for triphenyl phosphate.

[j] Theoretical P_2O_5 content 56.3%, upon basis of formula $C_2O_5H_2PO_4$ for monoethyl orthophosphate

[k] An untreated aqueous aliquot gave 8.75% P_2O_5 through molybdate precipitation, as indicative of the amount in the ionised ortho form.

To determine the phosphorus content of organics by means of the conventional molybdate precipitation, that content must be converted into the ionized ortho form through prefatory treatment known to be *specifically* adequate. Although the described analytical technique was found applicable for the exact determination of the phosphorus content of the particular hexaethyl tetraphosphate used in the greenhouse studies, and for a like determination upon certain other organic phosphates, that technique did not induce absolute ionization of the phosphorus content of the tricresyl and triphenyl phosphates. Until the evolution of an analytical procedure that will induce complete ionization of the phosphorus content of all organic phosphates, it seems imperative that each new product be analyzed by a procedure of proved specific applicability and adequacy.

NOTE: Subsequent to the submittal of the foregoing findings, the following notation was carried by *Chemical and Engineering News*, p. 2926, October 6, 1947:

> "The compound prepared by the reaction of triethyl phosphate and phosphorus oxychloride and sold commercially as "*hexaethyl tetraphosphate* does not, according to J. W. Hansen, California Spray Chemical Corporation, Richmond, exist in this form, but is a fortuitous mixture of organic phosphates which has an average molecular weight and combustion analysis percentages agreeing with the assigned formula. Chemical analyses of fractions obtained by distillation have shown that the active agent is a tetraethyl phosphate, probably tetraethyl peroxydiphosphate."

A PROCEDURE FOR THE DETERMINATION OF THE "AVAILABLE" MAGNESIUM ENGENDERED IN MIXTURES OF SUPERPHOSPHATE WITH OLIVINE, SERPENTINE, MAGNESITE, AND THEIR CALCINES*

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The role of magnesium in plant nutrition has been the objective of much research and the importance of this element as a fertilizer component is being accorded increasing recognition (6, 7, 12, 14, 27, 28, 37, 40). The soils of extensive areas are deficient in magnesium and this deficiency is registered by crops through visual symptoms that are well known. With recognition of the need for nutrient magnesium, the manufacture of magnesium-fortified fertilizers was a logical sequence.

PREVIOUS PRACTICES

The inclusion of dolomite as a source of nutrient magnesium in phosphatic fertilizers, and as a conditioner and neutralizer, was begun industrially in 1919, upon recommendation of the Tennessee Experiment Station. The P_2O_5 transitions that are induced by such inclusions and by admixtures of selectively calcined dolomite were dealt with in several papers (10, 17, 18, 24, 26). When the fertilizer effectiveness of dolomited phosphate had been demonstrated, and after Garner's classical experiments had established the need for magnesium in the growing of tobacco (7), the practice of the inclusion of dolomite in mixed fertilizers became widespread in the South Atlantic Coast States. Some 15 years ago, however, the "tobacco fertilizer group" contended that an adequate supply of nutrient magnesium was not developed with sufficient rapidity from included dolomite, and that tobacco fertilizers should contain magnesium sulfates.

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* A study conducted in the Cooperative Chemical Research Laboratory of the Department of Chemical Engineering, Tennessee Valley Authority, at the Tennessee Agricultural Experiment Station.

† Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 20–22, 1947.

Substantial proportions of "available" magnesia may be engendered when adequately moistened mixtures of acidic phosphates and finely ground dolomite are aged. In the formulation of higher analysis mixtures, however, the admissible quantity of included dolomite does not provide an adequate percentage of "available" magnesium. This difficulty would be overcome by the use of a low-cost magnesic material of such form and concentration that its inclusion in "high analysis" goods will meet the requirements for nutrient magnesium.

In correspondence with one of the present authors, the late G. H. Holford revealed that, because of the scarcity of dolomite in New Zealand, the mineral magnesium silicates were tried as conditioners of superphosphate. The resultant mixtures were of good physical condition and chemical composition and proved to be effective fertilizers. According to Andrews (1), the early New Zealand practice of neutralization of the H_3PO_4 of superphosphate by 8 to 10 per cent additions of dunite ($MgSiO_4$) was superseded by the use of serpentine $(Mg, Fe)_3Si_2O_7 \cdot 2H_2O$. The attendant reactions were dealt with by Askew (2), who concluded that $Ca_3(PO_4)_2$ was the preponderant end-product in the mixtures and that the "available" P_2O_5 content was not lessened. Similar benefits had been claimed in an early patent (31).

Extensive formations of olivine and serpentine occur in Western North Carolina and Northern Georgia and have been mapped through surveys conducted by the State of North Carolina in collaboration with the Tennessee Valley Authority (9). When incorporated into soils directly in amounts beyond those requisite for saturation with magnesium, a mineral magnesium silicate was found effective (27), but in more recent pot culture studies the initial crop gave virtually no response to incorporation of olivine at conventional liming rates. Findings by the present authors (15) served to demonstrate that adequate contents of "available" magnesium were generated in moistened mixtures of concentrated superphosphate with olivine and with serpentine, and without diminution in P_2O_5 availability.

PREVIOUS ANALYTICAL PROPOSALS

When calcined Kieserite was included in mixed fertilizers to provide immediately available magnesium, there arose the demand for an analytical procedure for the determination of water-soluble magnesium content. Pursuant to a request by the tobacco fertilizer group, L. G. Willis (38) proposed an analytical procedure to register that content. It was found, however, that the proposed aqueous extraction did not effect full recovery of the magnesium that was inmixed as magnesium sulfate. Moreover, a fertilizer fortified with MgO, or with selectively calcined dolomite, might be virtually devoid of water-soluble magnesium before analysis and yet the stipulated aqueous extraction would register a substantial percentage of magnesium as being in water-soluble form, because of the magnesium phosphates that develop during analysis (18).

Several helpful analytical procedures have been proposed for the determination of the "available" magnesium contained in mixed fertilizers (11, 30, 29, 33, 34, 35, 36). An acceptable procedure should effect full recovery of the magnesium present in the "available" forms of oxide, hydroxide, carbonate, sulfate, and phosphate, without appreciable concomitant dissolution of any incorporated magnesic or dolomitic materials, the chemical nature and particle size of which would delimit their reactivity in mixed fertilizers, even though such materials undergo disintegration slowly after incorporation into the soil and thus ultimately afford nutrient magnesium. However, the only official procedure for the determination of the magnesium carried by mixed fertilizers is the one prescribed for the acid-soluble content (3).

OBJECTIVE

The objective of the present study was to develop a much-needed method for the determination of the magnesium present as "available" in magnesium-fortified fertilizers. The designation "available" is deemed to include magnesium content attributable to mono-, di-, trimagnesium phosphates, to magnesium ammonium phosphate, to the oxide, hydroxide, carbonate, or sulfate. Excluded from that designation would be the component magnesium of materials whose inactivity in adequately moist phosphatic mixtures indicate that magnesium nutrition to plants would be slight and delayed.

EXPERIMENTAL

The several magnesium materials that were admixed with the concentrated superphosphate and the mixtures of Tables 1 and 2 were used in the exploratory analyses that led to the present proposal. The reactions that occurred in those mixtures are indicated by the following equations:

$$(1)\quad CaH_4(PO_4)_2\cdot H_2O + MgSiO_3 + 3H_2O \rightarrow CaHPO_4\cdot 2H_2O + MgHPO_4\cdot 3H_2O + SiO_2$$

$$(2)\quad CaH_4(PO_4)_2\cdot H_2O + MgCO_3 + 3H_2O \rightarrow CaHPO_4\cdot 2H_2O + MgHPO_4\cdot 3H_2O + CO_2\,.$$

The several reagents and techniques used in the early digestions of 1-gm charges, and comments upon the results, are summarized as follows:

1. *With 1 per cent citric acid—direct digestion*

(a) 30 minutes, 100 ml @ 30°C.

This solution was found to exert little effect upon separate 1-gm.

TABLE 1.—*Comparison of P_2O_5 transitions and engendered $MgHPO_4 \cdot 3H_2O$ in mixtures*[a] *of Wilson Dam triple superphosphate with olivine, serpentine, and magnesite*

CODE	T.S.P. MIXTURE	P_2O_5 ANALYSIS[b]				MONOCALCIUM PHOSPHATE				MgO IN MIXTURE	MgO AS W.S.[c]	MgO AS PHOSPHATE	
		TOTAL	W.S.[c]	C.I.[d]	AVAIL.	INITIAL CONTENT[e]	FINAL CONTENT[f]	CONVERSION				COMPUTED[g]	CONVERTED[h]
								ACTUAL	OF INITIAL				
		%	%	%	%	%	%	%	%	%	%	%	%
Olivine													
P-1265	1+1	23.00	5.20	0.85	22.15	39.38	4.74	34.64	88.0	23.58	1.42	5.54	23.5
P-1266	4+1	37.25	16.10	1.40	35.85	55.00	21.68	33.32	60.6	9.11	2.20	5.32	58.4
P-1267	9+1	42.25	25.30	1.50	40.75	63.50	39.40	24.10	38.0	4.67	1.75	3.86	82.6
Serpentine													
P-1271	1+1	24.25	5.50	0.90	23.35	37.25	4.58	32.67	87.7	16.34	1.66	4.75	22.9
P-1272	4+1	38.00	18.00	1.40	36.60	57.10	23.78	33.24	58.4	7.09	2.67	5.33	75.1
P-1273	9+1	44.00	28.60	1.55	42.45	69.50	42.64	26.86	38.6	3.56	2.56	4.12	89.8[i]
Magnesite													
P-1277	1+1	24.75	12.90	0.90	23.85	37.10	20.75	16.35	44.0	21.65	0.68	2.62	12.1
P-1278	4+1	39.50	26.90	1.40	37.10	55.88	33.28	22.60	40.4	8.36	1.42	3.61	43.2
P-1279	9+1	43.25	34.10	1.35	41.90	67.40	53.65	13.75	24.0	4.18	2.19	2.39	57.1

[a] Mixtures of 20-mesh T.S.P. and 100-mesh magnesic materials in the proportions indicated were moistened to 10 per cent H_2O content and cured at 30°C. After 4 week analyses (unreported) showed appreciable P_2O_5 transitions and, in some cases, the values were comparable to those shown for the 10-week period.

[b] Upon 50-mesh samples.

[c] 1 gm. leached with 250 ml. H_2O at room temperature.

[d] A.O.A.C. neutral citrate digestion.

[e] Computed from water-soluble P_2O_5 content of T.S.P.

[f] Final water-soluble P_2O_5 content corrected for $CaH_4(PO_4)_2 \cdot H_2O$ equivalence of water-soluble MgO.

[g] From equation: $CaH_4(PO_4)_2 \cdot H_2O + MgSiO_3 + 3H_2O \rightarrow MgHPO_4 \cdot 3H_2O + CaHPO_4 \cdot 2H_2O + SiO_2$.

[h] Of the total MgO in the mixture.

[i] [illegible]

TABLE 2.—*Comparison of P_2O_5 transitions and engendered $MgHPO_4 \cdot 3H_2O$ in mixtures*[a] *of Wilson Dam triple superphosphate with calcines of olivine, serpentine, and magnesite*

CODE	T.S.P. MIXTURES	P_2O_5 ANALYSIS[b]				MONOCALCIUM PHOSPHATE				MgO IN MIXTURE	MgO AS W.S.[c]	MgO AS PHOSPHATE	
		TOTAL	W.S.[c]	C.I.[d]	AVAIL.	INITIAL CONTENT[e]	FINAL CONTENT[f]	CONVERSION				COMPUTED[g]	CONVERTED[h]
								ACTUAL	OF INITIAL				
		%	%	%	%	%	%	%	%	%	%	%	%
Cal. Olivine													
P-1268	1+1	23.25	5.90	0.85	22.40	35.48	4.97	30.50	85.9	23.54	1.76	4.88	20.5
P-1269	4+1	37.00	18.00	1.40	35.60	55.45	23.03	32.40	58.4	8.16	2.85	5.28	64.6
P-1270	9+1	43.00	29.90	1.60	41.40	67.40	47.00	20.40	30.2	4.57	1.94	3.26	71.3
Cal. serpentine													
P-1274	1+1	23.50	5.50	0.80	22.70	36.20	5.22	31.00	85.6	20.57	1.28	4.95	24.0
P-1275	4+1	37.25	16.40	1.35	35.90	55.90	25.00	30.90	55.3	8.41	1.31	4.94	58.7
P-1276	9+1	41.50	24.10	1.55	39.95	65.30	36.35	29.00	44.0	4.43	2.05	4.63	104.5
Cal. magnesite, 750° C.													
P-1280	1+1	23.25	2.00	0.85	22.40	34.80	0.95	35.85	97.3	22.82	0.83	5.41	23.6
P-1281	4+1	35.50	3.80	1.50	34.00	53.04	2.52	50.52	95.2	9.50	1.35	7.07	74.4
P-1282	9+1	41.75	22.80	1.55	40.20	62.40	33.53	28.87	44.3	4.85	2.21	4.60	94.9
Cal magnesite, 950° C.													
P-1283	1+1	22.25	2.10	0.90	21.35	32.62	1.97	30.65	93.9	21.60	1.13	4.90	22.7
P-1284	4+1	36.00	4.60	1.50	34.50	53.93	3.41	50.52	93.6	8.90	1.52	7.07	79.4
P-1285	9+1	41.75	22.12	1.50	40.25	65.64	34.40	31.24	47.4	4.86	2.61	4.96	102.0

[a] Mixtures of 20-mesh T.S.P. and 100-mesh calcined magnesic materials in the proportion indicated were moistened and cured at 30°C. (P-1268 to P-1270 and P-1274 to P-1276 were moistened to 10 per cent H_2O content, P-1280 to P-1285 to 20 per cent content). After 4 weeks, analyses (unreported) showed appreciable P_2O_5 transitions and, in some cases, the values were comparable to those shown for the 10-week period.

[b] Upon 50-mesh samples.

[c] 1 gm. leached with 250 ml. H_2O at room temperature.

[d] A.O.A.C. neutral citrate digestion.

[e] Computed from water-soluble P_2O_5 content of T.S.P.

[f] Final water-soluble P_2O_5 content corrected for $CaH_4(PO_4)_2 \cdot H_2O$ equivalence of water-soluble MgO.

[g] From equation: $CaH_4(PO_4)_2 \cdot H_2O + MgSiO_3 + 3H_2O \rightarrow MgHPO_4 \cdot 3H_2O + CaHPO_4 \cdot 2H_2O + SiO_2$.

[h] Of the total MgO in the mixture.

charges of the magnesic minerals, but it did not dissolve like charges of $MgHPO_4 \cdot 3H_2O$, and hence this reagent was rejected.

2. *With 1 per cent ammonium citrate, pH 4.0—direct digestion*

	Minutes	*Volume*	*Degrees C.*
(a)	30	100 ml	30
(b)	60	100 ml	30
(c)	30	100 ml	50
(d)	30	100 ml	30
(e)	60	200 ml	30

3. *With 1 per cent ammonium citrate,* pH *4.0—extraction and digestion*

(a) Leached with 100 ml of reagent at room temperature and residue digested through end-over-end agitation 30 minutes with 100 ml @ 30°C.

(b) Ditto, 60-minute agitation

(c) Ditto, 30-minute agitation, at 50°C.

When used under the several conditions listed under 2 and 3 the acidulated ammonium citrate reagent effected complete dissolution of a 0.95-gm charge of $MgHPO_4 \cdot 3H_2O$, a 1-gm. charge of MgO, and a 0.90-gm charge of $MgNH_4PO_4 \cdot 3H_2O$, yet it registered only meager reactivity upon 1-gm charges of the magnesic minerals. However, when the conditions detailed under 2 and 3 were imposed upon the phosphatic mixtures of Tables 1 and 2, the acidulated 1 per cent ammonium citrate reagent did not effect complete recovery of the P_2O_5 present as "available," and therefore did not dissolve all of the $MgHPO_4 \cdot 3H_2O$ that had been engendered in those mixtures.

4. *With 2 per cent ammonium citrate,* pH *4.0—direct digestion*

The more concentrated reagent also effected complete dissolution of 1-gm. charges of MgO, dimagnesium phosphate, and magnesium ammonium phosphate, and likewise was relatively inert upon the magnesium minerals. Nevertheless, this reagent did not effect complete recovery of the "available" P_2O_5 content of the phosphatic mixtures of Tables 1 and 2, and hence was rejected.

5. *With 2 per cent citric acid—direct digestion*

Charges were digested as in a, b, c, d, and e of procedure 2, and also after being leached with 100 ml of the reagent at room temperature, as in a, b, and c of procedure 3.

The 2 per cent citric acid reagent was tested for its capacity to dissolve the magnesium content of several representative magnesic materials and the magnesium phosphate content of the mixtures of Tables 1 and 2, under the conditions specified in a, b, c, d, and e of procedure 2, and in a, b, and c of procedure 3. Upon basis of the analytical results, the use of the 2 per cent citric reagent, with prefatory leaching and digestion with continuous agitation 30 minutes at 50°C., was decided upon as being the only procedure found to meet all requirements.

THE PROPOSED EXTRACTION PROCEDURE

The foregoing observations indicate the progressively severe conditions, including trials with more concentrated solvents, that were imposed throughout the studies. The severe conditions of the exploratory studies were applied until it was found that the "available" P_2O_5 values obtained through the technique of the proposed procedure were in accord with those registered by the "official" method (3). This criterion was adopted for two reasons. Obviously the determination of phosphorus is more expeditious than that of magnesium and the values for the P_2O_5 recoveries register the extent to which the engendered magnesium phosphates undergo dissolution. After the effectiveness of the procedure had been indicated through the P_2O_5 determinations, the applicability of the procedure was substantiated through direct determinations of the extracted magnesium.

Although $MgHPO_4 \cdot 3H_2O$ is appreciably dissolvable by water, that compound is not removed quantitatively by the aqueous extraction prescribed as "official" for the removal of water-soluble P_2O_5. This serves to explain the differences between the values obtained by the direct determination of the MgO content of the 250-ml leachings of 1-gm charges of Table 3 and the corresponding values that were computed from the determined phosphate transitions that occurred in the mixtures of Tables 1 and 2. Transition to di-magnesium phosphate was computed upon basis of the difference between initial and final water-soluble P_2O_5 values, with appropriate correction for the P_2O_5 associated with the magnesium that was extracted concurrently from the engendered magnesium phosphate.

As the result of the findings obtained in the several approaches, the following technique is proposed:

Leach a 1-gm charge of a 50-mesh sample with 100 ml of 2% citric acid at room temperature, by means of successive decantations thru a Shimer filter. Digest the residue and filter pad with an additional 100 ml of the reagent in a 250-ml fertilizer flask 30 min. at 50°C., by means of the end-over-end rotator in a constant-temperature apparatus (16). Filter by suction and wash the residue five times with 5-ml portions of water at 65°C. Combine this digestate with the leachate in a 250-ml fertilizer flask, dilute to volume, and mix. Transfer a 10- or 25-ml aliquot of this soln into a 400-ml beaker; separate calcium content by double precipitation as oxalate, and determine the extracted magnesium by the conventional magnesium ammonium phosphate procedure. Pertinent suggestions and precautions for that determination will be included under "discussion."

REAGENT CAPACITY FOR THE COMPLETE DISSOLUTION OF THE ENGENDERED MAGNESIUM PHOSPHATES

As noted, the primary objective of the analytical studies was to develop a procedure whereby engendered magnesium phosphates and other magnesium compounds of recognized "availability" would be extracted quantitatively from fertilizer mixtures, but with meager dissolution of any residues of the admixed magnesic materials.

TABLE 3.—*"Available" magnesia attributed to engendered phosphates in mixtures of concentrated superphosphate and various magnesic materials, as determined by the proposed procedure and as computed from phosphate transitions*

MIXTURES	MgO OCCURRENCES IN THE MIXTURES			
	AS ACID-SOLUBLE[a]	IN WATER-EXTRACTS[b]	COMPUTED AS $MgHPO_4 \cdot 3H_2O$[c]	AS 2% CITRIC ACID-SOLUBLE[d]
	per cent	*per cent*	*per cent*	*per cent*
T.S.P. with:				
Olivine				
1+1	23.58	1.42	5.54	5.69
4+1	9.11	2.20	5.32	5.56
9+1	4.67	1.75	3.86	3.78
Calc. olivine				
1+1	23.54	1.76	4.88	5.83
4+1	8.16	2.85	5.28	5.29
9+1	4.57	1.94	3.26	2.92
Serpentine				
1+1	16.34	1.66	4.75	5.45
4+1	7.09	2.67	5.33	5.13
9+1	3.56	2.56	4.27	3.42
Calc. serpentine				
1+1	20.57	1.28	4.95	9.88
4+1	8.41	1.31	4.94	6.41
9+1	4.43	2.05	4.63	4.25
Magnesite				
1+1	21.65	0.68	2.62	3.87
4+1	8.36	1.42	3.61	2.83
9+1	4.18	2.19	2.39	2.06
Calc. magnesite[e]				
1+1	22.82	0.83	5.41	22.73
4+1	9.50	1.35	7.07	9.52
9+1	4.85	2.21	4.60	4.65
Calc. magnesite[f].				
1+1	21.60	1.13	4.90	20.99
4+1	8.90	1.52	7.07	8.96
9+1	4.86	2.61	4.96	4.99

[a] By A.O.A.C. procedure.

[b] Charge of 1 gm leached with 250 ml. H_2O (approximately 5 times the amount necessary to dissolve the maximal $MgHPO_4 \cdot 3H_2O$ content of the samples).

[c] Computed from the P_2O_5 transitions.

[d] Charge of 1 gm was leached with 100 ml 2 per cent citric acid and the residue was digested in additional 100 ml 2 per cent citric acid, with end-over-end agitation 30 minutes at 50°C.

[e] At 750°C. Virtually all of the magnesium content was as MgO.

[f] At 900°C. Virtually all of the magnesium content was as MgO.

A sample of each of the 21 experimental mixtures of Tables 1 and 2 was extracted by the proposed procedure to establish its capacity to effect complete dissolution of engendered magnesium phosphates. The resultant values for "available"P_2O_5 content were in accord with those registered by the "official" neutral citrate digestion procedure. There were twelve minus variations in range between 0.02 per cent and 0.70 per cent and eight plus deviations in range between 0.05 per cent and 0.37 per cent. The mean of the the minus deviations was 0.34 per cent and 0.37 per cent for the plus values, with an average deviation of minus 0.06 per cent.

Since the proposed 2 per cent citric acid dissolved all of the contained P_2O_5, other than that in the residues of raw rock, it follows that it also effected complete dissolution of the engendered magnesium phosphates. This was substantiated by the parallels of direct determination of the magnesium content of the extracts.

THE ANALYTICAL DETERMINATION OF THE MAGNESIUM OF THE 2 PER CENT CITRIC ACID EXTRACTS

The following precautionary measures were taken in the determination of magnesium carried by the 2 per cent citric acid extracts:

1. The aliquot should be such that its contained calcium will be as little as possible, but its magnesium content should be such as to assure a suitable precipitate of magnesium ammonium phosphate, regardless of the proportion of calcium. Aliquots of 10 or 25 ml from a 250-ml extract of a 1-gm charge are recommended, since the magnesium contained therein was found to produce a calcine of from 20 to 50 mgm of pyrophosphate.

2. The initial precipitation of calcium was made by the oxalic acid-ammonium oxalate procedure. It is important that the precipitation be made in a solution of near 200 ml that contains the NH_4Cl formed by the neutralization of at least 10 ml of concentrated HCl. The second calcium precipitation was made in similar manner from a solution of 150–175 ml.

3. After filtration of the second percipitate of calcium-oxalate, the ammonium salts contained in the combined filtrates were metathesized with HNO_3 and the resultant residue was brought into dilute HCl solution. Necessity for removal of iron and aluminum is obviated through the addition of 2 to 3 grams of citric acid to that solution prior to the magnesium ammonium phosphate precipitation. In case high degree of accuracy is essential, any appreciable manganese content should be removed. To assure that the precipitating reagents effect a complete throw-down of magnesium ammonium phosphate in the initial precipitation, 25 ml of concentrated NH_4OH should be added and a quiescent period of 30 minutes should be allowed, after which the solution should be stirred vigorously and then allowed to stand 4 hours before filtration.

4. The incomplete precipitation of calcium in the oxalate procedure would induce an inadmissible contamination in the subsequent magnesium ammonium phosphate precipitate. Such a contamination would be serious, especially when the solute magnesium is relatively low. The $Mg_2P_2O_7$ calcines from 14 representative determinations by the prescribed procedure were examined for this source of error. In every instance, absence of calcium was indicated by the ammonium oxalate test and also by Wolf's photometric method (39).

5. Other sources of error in the determination of magnesium is through loss as a co-precipitate in the calcium oxalate precipitation and its occlu-

sion by the calcium oxalate precipitate. Therefore, twelve of the second calcium oxalate precipitates were analyzed by the modified Stolberg procedure (8) and were found devoid of magnesium.

6. The removal of solvated silicia from the citric acid extracts necessitates a long and tedious procedure. Hence, to ascertain whether such removal is essential for analytical accuracy, magnesium determinations were made upon the extracts of 12 samples, with and without prior removal of silica. The results demonstrated that the presence of solvated silica did not induce higher values for magnesium. Hence, the tedious step of silica removal is not included in the directions for the analysis of the 2 per cent citric acid extracts of ordinary fertilizer mixtures.

Oxidation of the citric acid of the extracts, and the dehydration of the silica content, was effected by means of digestions with HNO_3 and $HClO_4$. Although oxidation of the organic matter of the filtrates with $HClO_4$ alone is precarious, the operation can be effectuated safely by starting it with a mixture of HNO_3 and $HClO_4$ in the cold, gradually increasing the temperature, and then boiling until the oxidation is completed.

7. To prevent carbonization of the citrate during the metathesis of the filtrates prefatory to the precipitation of $MgNH_4PO_4$, the solution should not be allowed to go to dryness. In case of carbonization, however, reclarification of the solution can be effected by another addition of HNO_3 and further digestion.

Utility of the Proposed Procedure:

In the development of the proposed procedure, the primary objective was to determine the quantity of magnesium generated as "available" by concentrated superphosphate in its mixtures with the relatively insoluble magnesium minerals—olivine, serpentine, and the crystalline type of magnesite.

The citric acid extractions and digestions of olivine, serpentine, or magnesite served to establish the fact that the described procedure effected only meager dissolution of either of those three minerals, as such, or when they were components of the phosphatic mixtures. It should be noted, however, that the prescribed volume of the 2 per cent citric acid is capable of effecting complete dissolution of the magnesium that may be present as a residue of MgO in a 1-gm charge along with any magnesium phosphate content. The MgO values determined directly by the 2 per cent citric acid digestion procedure were usually in close agreement with the MgO values that were computed from corresponding phosphate-transition values. In general, the directly determined "available" MgO values were higher than those computed from the P_2O_5 transitions, but in most cases the differences were within tolerance. The occasional lack of concordance may have been caused by a slight dissolution of the residues of the minerals in the charges. It may be, however, that the differences between the MgO values obtained by the two approaches were due as much to cumulative errors in the computations based upon the P_2O_5 transitions as to any inadequacy inherent to the proposed procedure.

Upon basis of the agreement between the values that were computed for magnesium from the P_2O_5 transitions of Table 3 and the values obtained by the direct determination of the magnesium content of the extracts, the proposed extraction-digestion procedure appears well adapted to the determination of "available" magnesium content of such phosphatic mixtures as those of Tables 1 and 2.

Effect of the Proposed Procedure upon Undecomposed Dolomite Residues:

After it had been found suitable for the extraction of "available" magnesium compounds derived from the several magnesic materials, the ques-

TABLE 4.—*The applicability of the proposed leaching-digestion procedure*[a] *for the determination of the "available" magnesium content of several magnesic materials, dolomite, and their phosphatic mixtures*

MIXTURES	DETERMINED MgO VALUES		
	TOTAL	AVAILABLE BY THE PROPOSED PROCEDURE[a]	
		ACTUAL	OF THE TOTAL
	per cent	*per cent*	*per cent*
Mineral Material Separates			
Olivine, −100	49.30	1.34	2.7
Serpentine, −100	38.90	1.67	4.3
Magnesite, −100	45.80	2.55	5.5
Calc. olivine, −100	50.00	1.66	3.3
Calc. serpentine, −100	43.50	3.42	7.8
Calc. magnesite, −100	89.80	89.80	100.0
Dolomite (W. Va.), −100	21.25	12.55	59.0
−60+70	21.25	9.81	40.4
−50+60	21.25	8.97	42.2
−30+50	21.25	6.95	32.2
−20+30	21.25	4.90	23.0
Prepared Materials			
$MgHPO_4 \cdot 3H_2O$, C.P.	23.12	23.12	100.0
$MgNH_4PO_4$, Powd.	20.74	20.74	100.0
MgO, Powd.	99.00	99.00	100.0
Calc.-Serp.-Phos.[b]	21.27	21.02	98.8
Calc.-Olivine-Phos.[c]	16.38	16.25	99.2
$MgSO_4 \cdot 7H_2O$, Cryst.	16.36	16.36	100.0
Kieserite	23.50	23.50	100.0
Mixtures			
0.25 Dolomite, −100			
0.25 T.S.P.	10.59		
0.25 $CaHPO_4 \cdot 2H_2O$	(5.62)[d]	10.47	98.8
0.25 $MgHPO_4 \cdot 3H_2O$			

TABLE 4.—*Continued*

MIXTURES	DETERMINED MgO VALUES		
	TOTAL	AVAILABLE BY THE PROPOSED PROCEDURE[a]	
		ACTUAL	OF THE TOTAL
	per cent	*per cent*	*per cent*
0.25 Dolomite, −100 0.25 Quartz 0.25 $CaHPO_4 \cdot 2H_2O$ 0.25 $MgHPO_4 \cdot 3H_2O$	10.59 (5.62)[d]	10.01	94.5
0.15 Dolomite, −100 0.3 $MgHPO_4 \cdot 3H_2O$ 0.3 $CaHPO_4 \cdot 2H_2O$ 0.25 T.S.P.	10.73 (6.94)[d]	9.65	89.9
0.25 Dolomite, −60+70 0.25 T.S.P. 0.25 $CaHPO_4 \cdot 2H_2O$ 0.25 $MgHPO_4 \cdot 3H_2O$	10.59 (5.62)[d]	10.01	94.5
0.25 Dolomite, −60+70 0.25 Quartz 0.25 $CaHPO_4 \cdot 2H_2O$ 0.25 $MgHPO_4 \cdot 3H_2O$	10.59 (5.62)[d]	8.52	80.4
0.15 Dolomite, −60+70 0.3 $MgHPO_4 \cdot 3H_2O$ 0.3 $CaHPO_4 \cdot 2H_2O$ 0.25 T.S.P.	10.73 (6.94)[d]	8.62	80.3
0.3 $MgSO_4 \cdot 7H_2O$ 0.3 $MgHPO_4 \cdot 3H_2O$ 0.3 $CaHPO_4 \cdot 2H_2O$ 0.1 T.S.P.	11.85	12.12	102.3
0.75 g. P-1280[e] 0.25 g. MgO	41.79	41.22	98.6
Com. Fert., dolomited[f]	3.98	3.50	90.0

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[a] Charges of 1-gm. were leached with 100 ml. of 2% citric acid and the residues digested with 100 ml additional for 30 min. at 50°C.

[b] Commercial product.

[c] Wilson Dam product.

[d] The amount from sources other than dolomite.

[e] Three parts of the 1+1 mixtures of MgO and superphosphate, P-1280 of Table 1, were supplemented by 1 part of MgO, reagent grade.

[f] A "4-8-4" product.

tion was raised as to whether the procedure would register "availability" for the magnesium content of mixed fertilizers attributable to residues of dolomite, the coarse particles of which show limited dissolvability. Although the answer to that question was not a primary objective in the study of a procedure for the quantitative recovery of "available" magnesium engendered in mixtures of superphosphate and magnesium silicate minerals, certain exploratory and integrated studies afforded the findings that are presented in Table 4.

The data show that the prescribed reagent and procedure exerted (a) complete dissolution of MgO, of $MgSO_4 \cdot 7H_2O$, of Kieserite, of $MgHPO_4 \cdot 3H_2O$, of $MgNH_4PO_4$, alone and as inclusions, and near-complete extraction of the magnesium contained in fused Ca-Mg phosphates, (b) only slight dissolvent action upon the separate charges of olivine, serpentine, and crystalline magnesite. Moreover, the proposed procedure did effect differential dissolution of a representative dolomite, in relation to its particle size. This is shown by the limited attack upon charges of the $-20+30$-mesh separate alone (and as an inclusion in phosphate mixtures), and by the near-complete dissolution of the -100-mesh separate inclusion in the experimental phosphatic mixtures. Because of the foregoing findings and in view of the demonstrated dissolubility and rapidity with which 100-mesh dolomite undergoes disintegration in acidic soils, in contrast with the persistence of its larger separates (4, 5, 13, 19, 20, 21, 22, 23, 25, 32), it seems logical and equitable to accord "availability" to magnesium that fertilizers may contain as residues of dolomite fines that are extractable by the proposed procedure.

CONCLUSION

Through interpretation of the present data, and upon basis of related findings as to rate of reactivity of dolomite separates in fertilizers, and after incorporation into soils (9, 18, 4, 5), it is concluded that the proposed procedure provides the means for the direct and accurate determination of any contained magnesium that is entitled to classification as "available," in the chemical control evaluation of commercial fertilizers.

In presenting their findings, the authors hope that the procedure proposed for the determination of available magnesium content of fertilizers will be found applicable for that objective and that their findings and conclusions will be substantiated through collaborative studies.

REFERENCES

(1) **Andrew**, R. L., Reversion of Ca Superphosphate by Serpentine. *New Zealand Jour. Soc. Tech.*, 23-B, 208–209 (1942).

(2) **Askew**, H. O., The Course of the Reactions Occurring between Serpentine and Superphosphate. *New Zealand Jour. Sci. Tech.* 24-B, 128–134 (1942).

(3) *Association of Official Agricultural Chemists, Methods of Analysis*, 6th Ed., 35, 2.51, 2.53 (1945).

(4) Collins, E. R., and Speer, F. R., Decomposition of Dolomitic Limestone in Soils When Used as a Neutralizing Agent in Complete Fertilizers. *This Journal*, XXII, 142 (1939).

(5) Dawson, F. R., Snyder, E. F., Leighty, W. R., and Reid, F. R., Decomposition of Dolomitic Limestone in Soils when used as a Neutralizing Agent in Mixed Fertilizers. *Ibid.*, XXII, 137–141 (1939).

(6) Eisenmenger, W. S., and Kucinski, K. J., Magnesium Needs in Seed Plants. *Soil Sci.*, 63, 13–17 (1947).

(7) Garner, W. W., McMurtrey, J. E., Jr., and Morse, E. G., Sand Drown, a Chlorosis of Tobacco and other Plants, Resulting from Magnesium Deficiency. *Science*, 56, 341–342 (1922).

(8) Hillebrand, W. F., and Lundell, G. E. F., Applied Inorganic Analysis, 487 (1929).

(9) Hunter, Charles E., Forsterite and Olivine Deposits of North Carolina and Georgia. Bul. 41, N. C. Department of Conservation and Development (1941).

(10) Keenen, F. G., and Morgan, W. A., Rate of Dolomite Reactions in Mixed Fertilizers. *Ind. Eng. Chem.* 29, 197 (1937).

(11) Kuzmeski, J. W., Effect of Particle Size on the Solubility of Magnesium in Dolomite and Magnesic Limestone in 4 Per Cent Citric Acid Adjusted to *p*H 4.0 with Ammonium Hydroxide. *This Journal*, XXII, 147–150 (1939).

(12) Lea, G. F., and Smith, J. B., Availability Studies on Magnesium in Magnesic Limestones, Serpentine, Olivine, and Magnesium Ammonium Phosphate. *Amer. Potato Jour.* 15, 1, 4–10 (1938).

(13) MacIntire, W. H., Influence of Form, Soil Zone and Fineness of Lime and Magnesia Incorporations upon Outgo of Calcium and Magnesium. *Soil Sci.* 21, 377–391 (1926).

(14) MacIntire, W. H., Dolomite as a Fertilizer Supplement—Its Behavior and Control. *This Journal*, 16, 589–596 (1933).

(15) MacIntire, W. H., Hardin, L. J., and Johnson, H. S., Jr. Available Magnesia Engendered by Concentrated Superphosphate in Mixtures with Olivine, Serpentine, Magnesite, and Their Calcines. (Presented at the 112th Meeting of the Amer. Chem. Soc., Sept. 1947.)

(16) MacIntire, W. H., Hardin, L. J., and Meyer, T. A., Proposal for Modification of the Wagner Procedure and Its Adaptation for P_2O_5 "Availability" of Fused Tertiary Phosphates. *This Journal*, 30, Fig. 1, p. 161 (1947).

(17) MacIntire, W. H., Hardin, L. J., and Oldham, F. D. Phosphate Fertilizer Mixtures: Chemical Changes and Physical Effects Induced in Mixtures of Triple Superphosphate with Dry and Wetted Limestone and Dolomite. *Ind. Eng. Chem.*, 28, 711–717 (1936).

(18) MacIntire, W. H., Hardin, L. J., and Oldham, F. D., Behavior of a New Source of Available Magnesium for Phosphatic Fertilizers. *Ind. Eng. Chem.*, 30, 651–659 (1938).

(19) MacIntire, W. H., Sanders, K. B., and Shaw, W. M., The Availability of Hydrated Lime, Limestone, and Dolomite of Two Degrees of Fineness, with Supplements of Red Clover Hay, as Measured by Lysimeter Leachings. *Jour. Amer. Soc. Agron.*, 25, 285–297 (1933).

(20) MacIntire, W. H., and Shaw, W. M., The Disintegration of Limestone and Dolomite Separates as Influenced by Zone of Incorporation. *Soil Sci.*, 20, 403–417 (1925).

(21) MacIntire, W. H., and Shaw, W. M., Fixation of Calcium-Magnesium from Burnt Limes, Limestone, and Dolomite Incorporations in Two Soil Zones. *Soil Sci.*, 22, 109–121 (1926).

(22) MacIntire, W. H., and Shaw, W. M., Lime-Magnesia Ratios in Dolomitic

Limestone as Influencing Solution and Soil Reactions. *Jour. Amer. Soc. Agron.*, **22**, 14–27 (1930).

(23) MacIntire, W. H., and Shaw, W. M., The Influence of Season upon Disintegration of Limestone and of Dolomite in Soil. *Ibid.*, **22**, 272–276 (1930).

(24) MacIntire, W. H., and Shaw, W. M., Reactivity Between Dolomite and Superphosphate Components. *Ind. Eng. Chem.*, **24**, 1401–1409 (1932).

(25) MacIntire, W. H., Shaw, W. M., and Robinson, B., The Leaching Action of Rainwater upon Dolomite and Limestone Separates Incorporated with Quartz in Outdoor Lysimeters. *Soil Sci.*, **46**, 9–19 (1938).

(26) MacIntire, W. H., and Shuey, G. A., Chemical Changes in Mixtures of Superphosphate with Dolomite and with Limestone. *Ind. Eng. Chem.*, **24**, 933–941 (1932).

(27) MacIntire, W. H., and Willis, L. G., A Comparison of Silicates and Carbonates as Sources of Lime and Magnesia for Plants. *Jour. Ind. Eng. Chem.*, **6**, 1005–1008 (1914).

(28) Prince, A. L., Zimmerman, M., and Bear, F. E., The Magnesium-supplying Powers of Twenty New Jersey Soils. *Soil Sci.*, **63**, 1, 69–78 (1947).

(29) Rader, L. F., Zahn, K. V., and Whittaker, C. W., The Citrate Solubility of Dolomite of Varying Particle Size. *This Journal*, **XXII**, 180–189 (1939).

(30) ——, Comparison of Chemical Methods for Estimating the Availability of Magnesium. *Ibid.*, **XXIII**, 404–411 (1940).

(31) Reichalt, Bruno, U. S. Patent No. 943,460, Dec. 1909; Reissue, 13,540, March 1913; 1,071,795, September 1913.

(32) Shaw, W. M., Solubilities of Calcic and Magnesic Limestone in Neutral Normal Ammonium Acetate. *This Journal*, **24**, 244–249 (1941).

(33) Smith, J. B., Report on Magnesium in Fertilizers. *Ibid.*, **XIX**, 288–301 (1936).

(34) Smith, J. B., Report on Magnesium in Fertilizers. *Ibid.*, **XX**, 252–263 (1937).

(35) Smith, J. B., and Deszyck, E. J., Report on Magnesium in Fertilizers. *Ibid.*, **XXI**, 277–293 (1938).

(36) ——, Report on Magnesium in Fertilizers. *Ibid.*, **XXIV**, 270–280 (1939).

(37) Truog, E., Goates, R. J., Gerloff, G. C., and Berger, K. C., Magnesium Phosphate Relationships in Plant Nutrition. *Soil Sci.*, **63**, 19–25 (1947).

(38) Willis, L. C., Private Communication to W. H. MacIntire (1933).

(39) Wolf, Benjamin, Rapid Tests for Major Nutrients in Soils and Plants. G.L.F. Seabrook Farms (1946).

(40) Zimmerman, M., Magnesium in Plants. *Soil Sci.*, **63**, 1, 1–12 (1947)

THE DETERMINATION OF FLUORINE ATTRIBUTABLE TO SOIL INCORPORATIONS OF CALCIUM FLUORIDE*

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The 1945 Edition of the Book of Methods contains the first prescribed procedure for the determination of the florine content of soils (1). Until 1933, there had been no analytical technique for the titrative determination of the fluorine in concentrations so meager as those obtained in soil distillates. After the thorium-nitrate titration procedure had been developed by Willard and Winter (11), however, determination of the fluorine content of soil became mainly the problem of a complete transposition of that content into a distillate that could be titrated.

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The prefatory incineration and fixative treatment of soil charges for perchloric acid distillations was evolved mainly from studies conducted in the laboratory of the Association's Referee on Soils and Liming Materials (6, 8), and after proof of the inadequacy of fusions with $Na_2CO_3-K_2CO_3$ and incinerations with magnesic materials—peroxide, carbonate, nitrate. Passage of a current of steam throughout the perchloric acid digestion, as advocated by MacIntire, Shaw, and Hardin for purge of liberated NH_3 (9), also was adopted. That technique served to eliminate bumping during distillation and diminished by half the time requisite for the complete expulsion of the fluorine from the $HClO_4$ digestate. In a contribution based upon the findings in the Referee's laboratory, it was stated: "Prerequisites for the distillation of entire fluorine content of soils are (a) preparatory fixation of the element and its conversion to compounds that undergo dissolution during digestion and yield the element to the distillates, (b) elimination of 'volatiles' that would pass into the distillates and vitiate their titration, (c) proper temperature control, and absence of colloidal silica and the prevention of bumping during distillation" (7, p. 107).

In the original directions (1), the prescribed ratio of soil charge to admixed CaO_2 was 1 to 3. Fluorine-free peroxide was not always obtainable, however, and Clifford substituted $Ca(OH)_2$ in his directions for the fixation of fluorine in the prefatory incineration of organic materials (2). In a subsequent presentation, the present senior author noted the alternative adopted by Clifford and prescribed, "Mix the charge intimately with either the peroxide or the hydroxide of calcium in a nickel or a platinum crucible. Incinerate thoroughly in an electric furnace below 500°C., and then ignite 30 minutes at 900C°." (7, p. 108).

It was assumed that the hydroxide would be as efficacious as the peroxide in the prevention of fluorine escape during incineration of the soil charges and in the fixation of component fluorine in forms from which complete evolution would be effected by the $HClO_4$ distillation. The present contribution stems from studies as to the admissibility of $Ca(OH)_2$ as a substitute for the prescribed CaO_2, and as to appropriateness of calcination temperature above 500°C.

PRESENT STUDIES

When soils of known content of additive fluorine were subjected to the prefatory incineration with $Ca(OH)_2$, in lieu of CaO_2, then to 30-minute calcination at 900°C., and then to three successive 100-ml perchloric acid distillations, the recoveries of fluorine were only fractional and as little as 6 per cent of the quantity that had been incorporated as CaF_2 in pre-

TABLE 1.—*Comparison of three distillation procedures for recovery of fluorine from experimentally phosphated soils that contained added fluorides, with and without limestone and dolomite*

SOIL NO.	OVERALL INCORPORATIONS	ADDED FLUORINE	FLUORINE RECOVERIES FROM 1-GM. CHARGES		
			METHOD I[a]	METHOD II[b]	METHOD III[c]
			SINGLE DISTILLATION WITH $HClO_4$ AT 135°C.	DOUBLE DISTILLATION FROM $HClO_4$ AT 165° & 135°C.	DIRECT DISTILLATION FROM H_2SO_4 AT 165° AND $HClO_4$ AT 135°C.
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*
337	None	None	60	68	88
	Rock phosphate[d]				
338	Alone	64	62	133	163
339	plus limestone	64	81	109	163
423	plus dolomite	64	124	101	150
424	plus $MgCO_3$	64	120	109	150
	TriCa phosphate[e]				
425	Alone	None	58	85	94
426	plus CaF_2	1200	79	135	1250
427	plus CaF_2+L.S.	1200	129	170	1265
428	plus CaF_2+Dol.	1200	88	168	1288
	DiCa phosphate				
429	Alone	None	77	60	110
430	plus CaF_2	1200	82	119	1287
431	plus CaF_2+L.S.	1200	77	118	1265
432	plus CaF_2+Dol.	1200	71	129	1288
	$Ca(PO_3)_2$				
433	Alone	None	73	33	103
434	plus CaF_2	1200	113	124	1250
435	plus CaF_2+L.S.	1200	116	101	1254
436	plus CaF_2+Dol.	1200	116	97	1288
437	plus L.S.	None	55	33	103
438	plus L.S.	None	59	34	100
439	plus Dol.	None	81	38	100

[a] Aggregate of three successive 100-ml. distillates by $HClO_4$ from 900°C. 30 minute calcine of soil $+Ca(OH)_2$.

[b] Double distillation with $HClO_4$ soil at 165°C. +aliquot at 135°C. from calcine as in (a).

[c] From sulfuric acid distillation from unignited soil, without additive $Ca(OH)_2$ and with H_2SO_4 and $HClO_4$ distillation of an aliquot.

[d] The 2-ton per acre incorporations of rock phosphate were made 10 years previously and they enriched the soil by 64 p.p.m. of fluorine.

[e] The other phosphated soils had been enriched with eight incorporations of CaF_2 that gave a 1200 p.p.m. enrichment of fluorine in 10 years

vious years. The dependability of $Ca(OH)_2$ as a fixative agent for fluorine in the ashing of organics had been questioned by Cox, *et al.* (3), by Dahle (4), and by Clifford (2), who believed that "the danger of loss of fluorine . . . through 'wetting' by a fixative solution" could be eliminated through distillation with H_2SO_4 at 165°C. and a $HClO_4$ distillation of the evaporated distillate at 135°C., as proposed by Wichmann and Dahle (10).

Therefore, the 2-acid distillation was tested as to the recovery of fluorine from 10-gm charges of unignited soil and 0.0120-gm and 0.0240-gm additions of F as CaF_2. The distillations with H_2SO_4 were at 165°C. and the succeeding perchloric acid distillations of aliquots were at 135°C. This

TABLE 2.—*The effectiveness of the direct double distillation of fluorine-fortified*[a] *soils of known history in lysimeter experiments*

SOIL NO.	OVERALL INCORPORATIONS	FLUORINE ADDITIONS AND ANALYTICAL RECOVERIES					
		ADDED	LEACHED	RESIDUAL	FOUND	RECOVERY OF RESIDUAL FLUORINE[b]	
		lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*per cent*
337	None	—	—	—	176	—	—
	Rock phosphate						
338	Alone	128	5	123	306	130	106
339	plus limestone	128	5	123	306	130	106
423	plus dolomite	128	6	122	300	124	102
424	plus $MgCO_3$	128	7	121	300	124	102
	TriCa phosphate						
425	Alone	None	—	—	188	—	—
426	plus CaF_2	2400	56	2344	2500	2312	99
427	plus CaF_2+L.S.	2400	63	2337	2530	2342	100
428	plus CaF_2+Dol.	2400	64	2336	2576	2388	102
	DiCa phosphate						
429	Alone	None	—	—	220	—	—
430	plus CaF_2	2400	40	2360	2574	2354	100
431	plus CaF_2+L.S.	2400	38	2362	2530	2310	98
432	plus CaF_2+Dol.	2400	41	2359	2576	2356	100
	$Ca(PO_3)_2$						
433	Alone	None	—	—	206	—	—
434	plus CaF_2	2400	51	2350	2500	2294	98
435	plus CaF_2+L.S.	2400	35	2365	2508	2302	98
436	plus CaF_2+Dol.	2400	40	2360	2576	2370	100
437	plus L.S.	None	—	—	206	—	—
438	plus L.S.	None	—	—	200	—	—
439	plus Dol.	None	—	—	200	—	—

[a] Through 8 incorporations of CaF_2 that supplied 2400 pounds of fluorine per 2,000,000 pounds of soil.

[b] From the quantities computed as differences between the amounts of fluorine incorporated and those found in the rain-water leachings.

procedure effected 100 per cent recoveries of the two fluorine additions.

The comparisons were extended through the use of the four soils that had received rock phosphate at the 2-ton rate 10 years previously, and 12 other soils that had been enriched in fluorine through 8 incorporations of CaF_2 during the 10 years. The data of Table 1 register low fluorine recoveries from the 900°C. calcines of the mixtures of fluorine-enriched soils and $Ca(OH)_2$ by means of the single and double perchloric distillations, in contrast to the higher recoveries obtained by H_2SO_4 and $HClO_4$ distillations of the unignited soil.

The recoveries by the $HClO_4$ distillations from the soils that contained rock phosphate were erratic, but all recoveries were much larger percentagely than any recovery from the common input of CaF_2 that supplied more than 18 times the 64 p.p.m. enrichment that was supplied by the apatite of the single incorporation of rock phosphate.

The procedure used in the direct distillation of fluorine from unignited soil was as follows:

> A charge of either 0.5 gm or 1 gm of 325-mesh soil was distilled from 20 ml of fluorine-free H_2SO_4 (1+1) in a current of steam at 165°C., until a distillate of 250 ml was obtained. A 25-ml aliquot of that distillate then was likewise distilled at 135°C. with 15 ml of 60 per cent $HClO_4$ that contained 0.2 gm of Ag_2SO_4, to obtain a 100-ml distillate. The acidity of 20 ml of this distillate was determined by titration with 0.05 N NaOH and the acidity of the remaining 80 ml was adjusted to contain the equivalent of 4 ml of 0.05 N $HClO_4$. This sodium-free 80-ml aliquot then was titrated with $Th(NO_3)_4$ [0.20 gm $Th(NO_3)_4 \cdot 4H_2O$ per liter] that had been standardized against the double distillates obtained from known amounts of NaF, as prescribed.

TABLE 3.—*The effect of variance in proportion of $Ca(OH)_2$ in soil charge upon recovery of fluorine from calcines[a] of a slagged soil[b]*

SOIL	RATIO OF SOIL TO $Ca(OH)_2$ IN CHARGE	CALCINATION OF CHARGE		FLUORINE RECOVERY[c]
		TEMP.	DURATION	
		°C.	*Minutes*	*p.p.m.*
Alone	None	—	None	1108
With $Ca(OH)_2$	1:0.5	900	30	63
With $Ca(OH)_2$	1:2.0	900	30	150
With $Ca(OH)_2$	1:7.0	900	30	875
With $Ca(OH)_2$	1:9.3	900	30	925

[a] Of 0.5 gm. of soil.
[b] One that had received incorporations of quenched calcium silicate slag 6 years previously.
[c] By double (H_2SO_4 and $HClO_4$) distillations.

The 2-acid distillation procedure then was checked further for accuracy and for reproducibility of results through the comparative analyses of a series of fluorine-enriched soils, records of which had been kept in a 10-year lysimeter experiment. Respective incidences of fluorine were computed as the differences of the amounts incorporated and the quantities found in the leachings per annum. In terms of pounds of fluorine per 2,000,000 pounds of soil, and as percentage of the quantities computed to be residual from the incorporations of CaF_2, the analytical values of Table 2 demonstrate that fluorine content of soils is registered accurately by the direct distillation of unignited charges by H_2SO_4 and $HClO_4$ in succession, at respective temperatures of 165°C. and 135°C.

THE REASON FOR THE FRACTIONAL RECOVERIES BY THE $HClO_4$ DISTILLATES FROM THE CHARGES OF SOIL+$Ca(OH)_2$

An explanation then was sought as to why the recoveries of fluorine obtained by the $HClO_4$ distillations from calcined charges of lime-plus-soil were far from complete, as shown in Table 1, although full recoveries of additive fluorine had been registered by the $HClO_4$ distillations of soils incinerated with CaO_2 and ignited at 900°C., as reported by MacIntire and Hammond (8). It was assumed that the deficiency in recovery might be a question of (a) adequacy in the proportion of the fixative $Ca(OH)_2$ in the analytical charge and (b) the temperature and duration of the prefatory calcination.

TABLE 4.—*The effect of variance in the temperature, and in the duration, of calcination of soil+$Ca(OH)_2$ upon recovery of fluorine through double distillation*[a]

CALCINATION		FLUORINE RECOVERY[b]
TEMP.	DURATION	
°C.	*minutes*	*p.p.m.*
—	None	1250
500	5	1265
500	15	1288
500	30	1275
500	60	1275
500 and 900	60 and 5	150
500 and 900	60 and 15	25
500 and 900	60 and 30	25
500 and 900	60 and 60	0

[a] From H_2SO_4 at 165°C., 1-gm. charges of soil and $Ca(OH)_2$ [1 to 1], and from aliquot by $HClO_4$ at 135°C.

[b] Basis of moisture-free soil, No. 426 (Tables 1 and 2), which had received 8 inputs of CaF_2 to the aggregate of 1200 p.p.m. in 10 years.

To test its adequacy, the hydroxide fixative was admixed with a soil of high fluorine content in the four proportions listed in Table 3, and the respective mixtures were calcined 30 minutes at 900°C. It is obvious that all of the resultant recoveries of fluorine were fractional and that disparity between content and recovery became less as the proportion of $Ca(OH)_2$ per soil-charge was increased. But, even with a 1 to 9 ratio of soil to $Ca(OH)_2$—which is thrice the ratio of 1:3 that had proved adequate for additive CaO_2—the maximal recovery of fluorine from the 900°C. calcines did not equal the one obtained by the direct H_2SO_4 and $HClO_4$ distillation of the unignited soil. This disparity might be attributable to two factors, singly or jointly. The hydroxide may not have prevented the loss of fluorine during the prefatory incineration and calcination, or it may not have prevented the formation of those alumino combinations of fluorine that do not undergo dissolution and yield fluorine to the perchloric acid distillations.

The volatility of CaF_2 at 900°C., alone and in mixtures with quartz, was determined. When charges of 0.2 gm were heated alone 60 minutes at 900°C. and then distilled with $HClO_4$ at 135°C., fluorine recovery was complete. But when identical charges of CaF_2 were mixed with pulverized quartz and the mixture heated 60 minutes at only 500°C., the recoveries were only 39 per cent, as shown in Table 4. When the heating of companion charges at 500°C. was followed by 15-minute and 30-minute calcinations at 900°C., there were further losses of fluorine, up to 50 per cent of content. Obviously, an adequate proportion of a protective additive, such as CaO_2. or the hydroxide of calcium in much larger proportion, is essential to prevent volatilization of fluorine when an analytical charge of CaF_2 is heated to 900°C. in contact with quartz.

The near-recovery from the 900°C. calcine of soil with 9 parts $Ca(OH)_2$ serves to explain the disparities between full recoveries from the 500°C. calcines of the $Ca(OH)_2$-fortified charges and the fractional recoveries from corresponding 900°C. calcines.

An experimental soil of known history (No. 426 of Table 2) and a determined fluorine content of 1250 p.p.m. then was used in the four 500°C. heatings and those extended to 900°C., as detailed in Table 4. Each 1-gm charge of soil was impregnated with a slurry of calcium hydroxide that provided 0.3 gm of $Ca(OH)_2$. Four of the slurried charges were dried and calcined at 500°C. for periods of 5, 15, 30, and 60 minutes, and then subjected to the H_2SO_4 and $HClO_4$ distillation. Four identical charges were heated 60 minutes at 500°C. with further respective heating periods of 5, 15, 30, and 60 minutes at 900°C. The values registered by direct distillation of the unheated charge and by the distillations of the 500°C. calcines can be rated as identical. In contrast, the additional heatings at 900°C. caused progressive diminution in fluorine recoveries with increased duration, and even no recovery from the calcine that was subjected to the 60-minute experimental heating. Hence, when the 1 to 1 mixtures of soil and $Ca(OH)_2$ were calcined at 500°C. for periods between 5 and 60 minutes, the hydroxide proved adequate as a fixative that allowed full release of component fluorine in the double distillations.

SUMMARY AND CONCLUSIONS

A correlation of the earlier Referee studies with those now reported serves to establish the following conclusions.

Fluorine present as apatite in the soil is recoverable far more readily than fluorine supplied by inputs of CaF_2, when calcined charges of soil are subjected to distillation with either H_2SO_4 or $HClO_4$.

The prefatory incineration and calcination of soil charge with CaO_2, as now prescribed in the *Book of Methods*, has been found adequate to prevent loss of fluorine from the analytical charge and to assure full release of fluorine content by the prescribed perchloric acid distillation in a current of steam. Also, the further calcination at 900°C. was proved unnecessary, and even unadmissible.

Used in the proportion of at least 10 parts to one part of soil, $Ca(OH)_2$ can be substituted for CaO_2 in calcination at 900°C., but such large proportion and high temperature are not advocated.

The findings establish the adequacy of $Ca(OH)_2$ as a fixative for the fluorine content of an equal weight of soil, when the calcination of the mixture is at 500°C. for a period so brief as 5 minutes.

It is established also that full recovery of fluorine content of rock-derived soils is effected by direct H_2SO_4 distillation of the charge of *unignited* soil at 165°C., with sequential $HClO_4$ distillation and with titration against a thorium nitrate solution, *standardized through identical double distillation*, as prescribed in the foregoing procedure.

Since it assures (a) full recovery of fluorine, (b) obviates the use of a fixative, (c) eliminates incineration and calcination, and (d) is more expeditious than the peroxide procedure, the direct distillation technique is deemed as meritorious and even preferable to the present accepted procedure, which prescribes that a mixture of soil and CaO_2 be calcined at 500°C., and further at 900°C., as prefatory to the distillation with $HClO_4$.

REFERENCES CITED

(1) Association of Official Agricultural Chemists, Official and Tentative Methods of Analysis, Sixth Edition, p. 13 (1935).
(2) Clifford, P. A., *This Journal*, **24**, 360 (1941).
(3) Cox, *et al.*, *Jour. Dental Research*, **18**, 486 (1939).
(4) Dahle, Dan, *This Journal*, **22**, 338–39 (1939).
(5) Dahle, Dan, and Wichmann, H. J., *Ibid.*, **19**, 313 (1936).
(6) Hammond, J. W., and MacIntire, W. H., *Ibid.*, **23**, 398–404 (1940).
(7) MacIntire, W. H., *Soil Sci.*, **59**, 105–09 (1945).
(8) MacIntire, W. H., and Hammond, J. W., *This Journal*, **22**, 231–36 (1939).
(9) MacIntire, W. H., Shaw, W. M., and Hardin, L. J., *Ind. Eng. Chem., Anal. Ed.*, **10**, 144–52 (1938).
(10) Wichmann, H. J., and Dahle, D., *This Journal*, **16**, 620 (1933).
(11) Willard, H. H., and Winter, O. B., *Ind. Eng. Chem.*, **5**, 7–10 (1933).

THE DETERMINATION OF TOTAL BORON IN PLANT MATERIAL WITH "CHROMOTROPE-B"*·†

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The role of boron in living plants is being investigated in this laboratory. To facilitate this study a rapid method for the determination of boron was required. A search of the literature revealed only three types of methods that might be applicable. These were examined critically.

The electrometric titration of boric acid used by Wilcox (8), McCormick (3) and others which requires 5–25 gm. samples lacks the sensitivity required. The Naftel procedure (6) was eliminated on the basis of previous work at this laboratory (4) and elsewhere (1). The quinalizarin method described in *Methods of Analysis, A.O.A.C.* (6th Edition) is subject to several disadvantages. Particularly objectionable are the hazardous use of fuming sulfuric acid in the process and the difficulty experienced in maintaining the critical sulfuric acid concentration. The above methods all require long ignitions at 450°C. or less. This is a serious disadvantage when a considerable number of samples are to be analyzed.

The addition of a base is reported unnecessary by the above workers. Piper (7), however, describes two different ignition procedures; in one sodium hydroxide is added to the sample and in the other calcium hydroxide. Both procedures entail complicated manipulative detail and are time consuming and inconvenient.

These disadvantages are reduced to a minimum in the method described. The ignition lasts for two hours and is made at 600° C. with barium hydroxide added to the sample. The reagent, p-nitrobenzeneazo-1, 8-dihydroxynapthalene-3, 6-disulfonic acid ("Chromotrope-B") is used as a 0.005 per cent solution in concentrated (95.5 per cent) sulfuric acid. Feigl (2) has recorded the use of the reagent for the detection of boron, but its use for the determination has not been previously reported.

METHOD

APPARATUS

Coleman Model 11 Universal spectrophotometer with ½ inch square cuvettes. In this determination no filter is used.

10 ml automatic burette protected from atmospheric moisture with calcium chloride tubes.

15 ml centrifuge tubes—pyrex glass tubes are satisfactory.

125 ml flat bottom boiling flasks of "Corning" Brand Alkali Resistant (boron free) glass.

Platinum dishes.

REAGENTS

Acetic acid—70 per cent.—Made by dilution of glacial acetic acid (Reagent grade).

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Concentrated sulfuric acid (Reagent grade, 95.5 per cent H_2SO_4).—"Baker's Analyzed" acid has been used satisfactorily without analysis.

Saturated solution of recrystalized barium hydroxide.

A 0.005 per cent solution by weight of p-nitro-benzenazo-1, 8-dihydroxynaphthalene-3, 6-dusilfonic acid (Eastman Organic Chemicals No. 4411), "Chromotrope-B" in concentrated sulfuric acid.

PREPARATION OF STANDARD CURVE

Dissolve 0.0930 g anhydrous $Na_2B_4O_7$ in several hundred ml of 70% acetic acid. Add 100 g of recrystallized $Ba(OH)_2 \cdot 8H_2O$ and allow to dissolve. Dilute to one liter with 70% acetic acid. This solution contains 20 mmg of boron per ml. Dilute portions of this stock solution so that a series of standards containing 0 to 15 mmg of boron per ml is obtained. Treat one ml of each solution as described below and determine per cent transmission at a convenient but reproducible temperature between 20° and 30°C. Draw a concentration vs. transmission curve from these data.

PROCEDURE

Place a 0.2–0.5 g sample of plant tissue (air-dried and 60-mesh) in a platinum dish. Determine the moisture content on a separate sample by drying for four hours at 100°C. Add 5 ml of a saturated soln of barium hydroxide and place the dish in a cool muffle furnace. Raise the temperature to 600°C. and ignite for two hours. Remove the dish from the furnace, cool, and add 5.0 ml of 70% acetic acid (10.0 ml may be necessary when large amounts of boron are present). Triturate with a rubber policeman and pour the suspension into a 15 ml centrifuge tube. After centrifuging transfer 1.0 ml of the clear supernatant liquid to a 125 ml flask (boron-free glass) and add 10.0 ml of the 0.005% solution of "Chromotrope-B" in concentrated sulfuric acid. Mix and allow the soln at least 30 min. for color development and for cooling to the same temperature used in preparing the standard curve. Using ½ inch square cuvettes, determine the per cent transmission at 620 $m\mu$ against a reference soln prepared similarly but with 1 ml of 70% acetic acid instead of the boron soln. (Contrary to the usual practice no filter is used while making transmission readings.) Determine micrograms of boron in the aliquot from the standard curve and calculate total boron in the sample by the equation:

$$\underset{\text{(dry basis)}}{\text{p.p.m. boron}} = \frac{\text{ml acetic acid added to ash} \times \text{micrograms boron found/ml}}{\text{oven dry weight of sample}}$$

Duplicate analyses can be easily completed in about three hours.

EXPERIMENTAL

Interfering Elements and Ions:

Tests were made on 28 cations and 21 anions for interference with the reaction. A small quantity (five to 10 milligrams) of a compound of the ion to be tested was added to the boron solution on the spot plate and the reagent added. Silver, lead, and strontium interfered somewhat with color development. With smaller amounts of these ions no interference could be detected. Several organic anions interfered by modification of the boron "Chromotrope-B" color, but as such ions are absent after ignition this is not significant. The inorganic anions NO_3, NO_2, Cr_2O_7, and SeO_4 interfere with the test by reacting with the reagent to form a red compound.

Fluorides prevent color development by forming the stable fluoboric acid or boron fluoride. No ion tested gave a false positive test.

Effect of Drying Procedure on Boron Recovery:

A sample of wild cherry leaves was collected, finely shredded, and well mixed. Random samples were taken from the main sample and treated as shown in Table 1. Analytical results are based on total dry matter.

TABLE 1.—*Boron recovery as influenced by pre-ignition drying*

TREATMENT	BORON FOUND (DRY BASIS)	RECOVERY
	p.p.m.	*per cent*
Air dried, 48 hours	22.9	100.0
Air dried, 48 hours then dried 3 hrs. at 100°C.	21.2	92.6
Dried 70° for 24 hours	19.9	86.9
Dried 4 hours (to constant weight) at 100°C.	19.8	86.5

Effect of Variations in Temperature of Ignition and in Base Added:

The procedure was the same as that described previously except that the ignition temperature and the base added were varied. Results obtained are shown in Table 2. The time of ignition was two hours.

TABLE 2.—*Effect of varying ignition temperature and base added on boron recovered from the synthetic sample (97.2 p.p.m. of boron) as anhydrous* $Na_2B_4O_7$

IGNITION TEMPERATURE	BASE ADDED	BORON FOUND
		p.p.m.
500°C. (placed in cool furnace)	None	11
500°C. (placed in cool furnace)	$Ba(OH)_2$ (satd. soln)	92.0
500°C. (placed in cool furnace)	K_2CO_3 (solid)	89.0
500°C. (placed in cool furnace)	K_2CO_3 (20% soln)	49.0
500°C. (placed in cool furnace)	$CaCO_3$ (solid)	69.0
600°C. (placed in cool furnace)	$Ba(OH)_2$ (satd. soln)	92.7
750°C. (placed in cool furnace)	$Ba(OH)_2$ (satd. soln)	89.5
900°C. (placed in cool furnace)	$Ba(OH)_2$ (satd. soln)	82.7
400°C. (placed in cool furnace)	$Ba(OH)_2$ (satd. soln)	87.8
600°C. (placed in cool furnace)	$Ba(OH)_2$ (solid)	89.8
600°C. (placed in hot furnace)	$Ba(OH)_2$ (satd. soln)	84.1

Similar results were obtained with kelp and bean seed samples. The highest result obtained was considered the most accurate, and in each case this was obtained with the 600° ignition.

There is some loss of boron on ignition of the sample. This is minimized in the recommended procedure. For example, the sample of kelp had been previously analyzed by the quinalizarin method and the boron content reported as 270 p.p.m. (5). This result is 14 per cent lower than the 314.5 p.p.m. of boron found by the method herein described.

Small variations in temperature of the solutions when reading per cent transmission are inconsequential. Variations of as much as 1 per cent in the concentration of the sulfuric acid do not affect results.

RESULTS

The results of anlyases of several plant samples and a synthetic sample containing 97.2 p.p.m. of boron as anhydrous $Na_2B_4O_7$ are shown in Table 3. A number of analyses were run on each sample. The average deviation of a single observation indicates the precision of the method.

TABLE 3.—*Results of Analyses*

SAMPLE	BORON ADDED	NUMBER OF DETERMINATIONS	MEAN BORON FOUND	AVERAGE DEVIATION OF SINGLE OBSERVATION	RECOVERY
	p.p.m.		*p.p.m.*	*p.p.m.*	*per cent*
Synthetic	97.2	9	92.7	1.1	95.4
Bean Seed		6	21.8	.4	
White Pine Needles (N)		5	38.0	1.1	
White Pine Needles (D)		5	42.6	1.1	
Wild Cherry Leaves (1)		4	17.1	.2	
Wild Cherry Leaves (2)		3	22.9	.4	
Kelp		6	314.5	2.8	

The color does not strictly obey Beer's law. It is, however, reproducible with different batches of the reagent solution. The standard curve is shown in Figure 1. The concentration versus per cent transmission curve for the quinalizarin method is also shown for comparative purposes.

SUMMARY

A method for the determination of boron in plant materials is proposed based on the colored complex or ester of boric acid and "Chromotrope-B" (p-nitrobenzeneazo-1, 8-dihydroxynapthalene-3, 6-disulfonic acid).

Oxidizing agents, nitrates, nitrites, and fluorides interfere with the reaction.

The method of drying the sample has been shown to have a significant effect on the amount of boron recovered.

The ignition of the sample is carried out at 600°C. after the addition of barium hydroxide. It is essential that the sample be placed in a cool furnace and the temperature then raised to 600°C.

Several plant samples as well as a synthetic sample have been analyzed by the method. In the synthetic sample, recovery was 95.4 per cent.

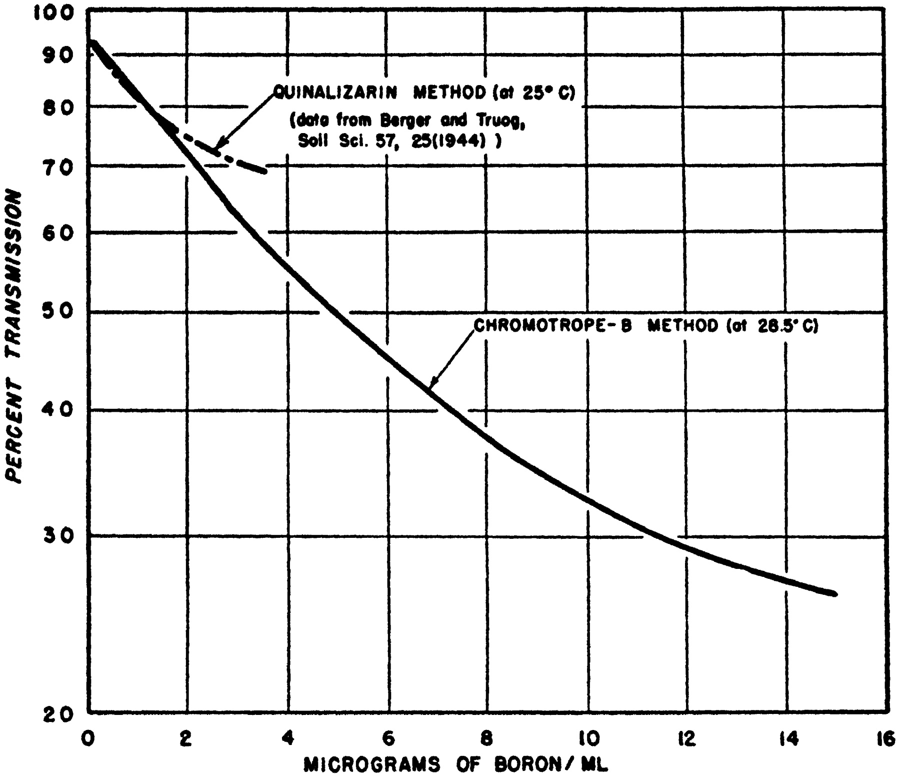

FIG. 1.—Concentration vs. per cent transmission for chromotrope-B and quinalizarin

The authors wish to express their appreciation to Dr. L. K. Wood of this Department for his helpful suggestions and criticisms in the preparation of this manuscript.

REFERENCES

(1) Arizona Agricultural Experiment Station. Fifty-third Ann. Rept., p. 16 (1942).
(2) FEIGL, F., *Qualitative Analysis by Spot Tests.* 2nd. English Ed. Translated by J. W. Mathews, p. 224. Nordmann Publishing Co., New York (1939).
(3) MCCORMICK, D. R., *Chemist-Analyst,* **31,** 35 (1942).
(4) MCHARGUE, J. S., and HODGKISS, W. S., *This Journal,* **25,** 311 (1942).
(5) MCHARGUE, J. S., HODGKISS, W. S., and OFFUTT, E. B., *J. Am. Soc. Agron.,* **32,** 622 (1942).
(6) NAFTEL, J. A., *Ind. Eng. Chem., Anal. Ed.,* **11,** 407 (1939).
(7) PIPER, C. S., *Soil and Plant Analyses.* Interscience Publishers (1944).
(8) WILCOX, L. V., *Ind. Eng. Chem., Anal. Ed.,* **12,** 341 (1940).

NOTES ON THE DISTILLATION OF AMMONIA IN THE KJELDAHL DETERMINATION. A NEW CONNECTING BULB*

By C. O. WILLITS, H. J. JOHN, and L. R. ROSS (Eastern Regional Research Laboratory,† Philadelphia 18, Pennsylvania)

In a study to modify the Kjeldahl method for nitrogen so that it would be suitable for all types of nitrogenous organic compounds, inconsistent "blank" values were obtained even when the same reagents were used. Since the nitrogen "blank" values should be constant for any set of reagents, this inconsistency could be accounted for only by assuming that droplets of the alkaline reaction mixture were entrained in the vapors and carried through the Kjeldahl connecting bulb. The same difficulty was experienced with several commonly used connecting bulbs which have either been described in the literature (1, 3, 4,) or listed as catalogue items. This "distillation" of nonvolatile alkali had been recognized previously by Davisson (1) and by Lovecy (4). The variation of the "blank" depends upon the efficiency of the bulb in removing droplets of entrained alkali from the vapor stream and upon the concentration of the droplets in the vapor caused by the reagent or by the rate of distillation. The blank, therefore, becomes a possibly serious source of error in the Kjeldahl method for the determination of nitrogen. Obviously, with a varying blank, the selection of the correct blank becomes impossible. It does not necessarily follow that reproducible blank values are correct, since the conditions of the distillation of the samples and blank may be different. To eliminate this nonvolatile alkali error and still permit complete recovery of ammonia, a new connecting bulb has been designed, and has been used satisfactorily during the past year as part of a 24-unit Kjeldahl apparatus in which a large number of nitrogen determinations were made.

Description of bulb.—The new connecting bulb (Fig. 1) is unique in that it has two wire screens, which serve as condensers to form water films at the beginning of the distillation, as plates to support the condensed water, and as diffusors of the distilled vapors.

The application of the countercurrent extraction principle to Kjeldahl connecting bulbs is not new. Davisson (1) made use of this principle, but his bulb failed in that it did not start to scrub the vapors until sufficient distillate had collected in the bulb to cover the holes in the small inner bulb. During this time any droplets that occurred in the vapor were free to be carried through the bulb by the vapor stream. Lovecy (4) also made use of this principle, but unless the distillation rate was kept slow the scrubbing action was not effective and the condensate was carried over into the receiver.

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† One of the Laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture.

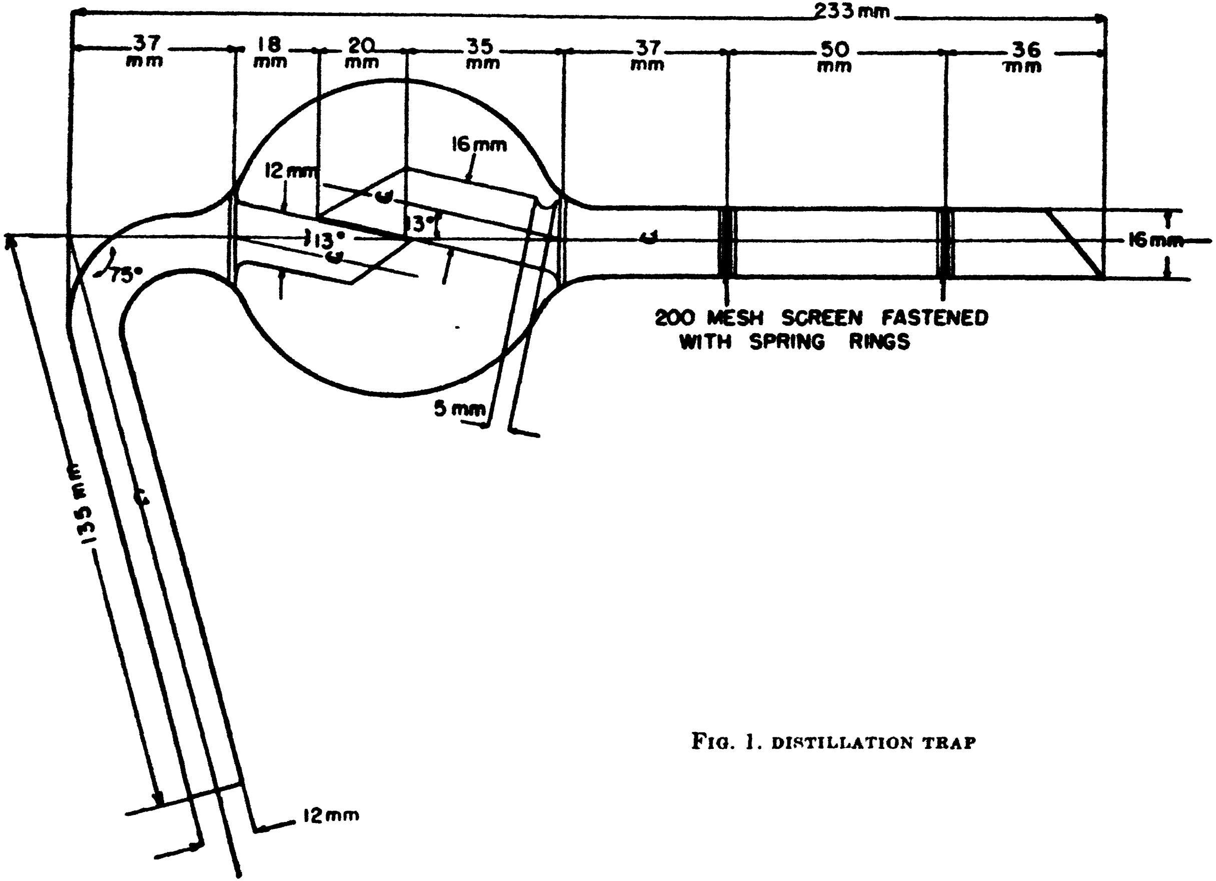

FIG. 1. DISTILLATION TRAP

The experimental connecting bulb scrubs all the vapors passing through it, since a water film forms on the wire screen plates the instant water vapor reaches them, and these films are maintained throughout the distillation. In passing through the layer of water condensate built up on the upper screen plate, the vapors carry water into the bulb vapor trap. The amount of condensate carried into the bulb seldom exceeds 35 ml., even with long distillation times.

The condensate in the bulb is maintained above *p*H 7 by the nonvolatile alkali scrubbed from the vapor, and it is kept at or near the boiling point by the passing steam. Under these conditions, no ammonia should be retained in the condensate in the connecting bulb. That no ammonia was retained was confirmed by collecting the bulb condensate at the end of a nitrogen determination and testing it with Nessler's reagent.

A comparison was made of four of the commonly used Kjeldahl connecting bulbs with the experimental bulb. The Lovecy bulb was not considered in these tests because of its fragile structure and because of the care that must be exercised in controlling the necessarily slow distillation rate.

The distillation mixture consisted of 100 ml of 50 per cent sodium hydroxide, which had been boiled to remove any ammonia, 250 ml of water and 10 grams of mossy zinc. Two hundred milliliters of distillate were collected in receivers containing 50 ml of 0.0100 *N* hydrochloric acid at the rate of 100 ml in 30 minutes for the slow distillation and 200 ml in 30 minutes for the rapid distillation. This test mixture, which is similar to that used by Davisson, produces a heavy spray rich in nonvolatile alkali. The results are given in Table 1.

The zinc was responsible for the large amounts of sodium hydroxide in the vapor, since when no zinc was used in the distillation mixture, the amount of sodium hydroxide carried over was small and nearly independent of the type of bulb used. The form of the zinc was also an important factor, since mossy zinc caused almost twice as much sodium hydroxide to be carried over as did an equal weight of 20-mesh zinc.

In these tests, those bulbs which did not provide for scrubbing the distilled vapors (the Hopkins, Iowa State and the Kjeldahl) passed the largest amount of sodium hydroxide. The poorest duplicate check results were obtained with the bulbs which passed the most sodium hydroxide. Of the nonscrubbing-type connecting bulbs the Hopkins bulb allowed the greatest amount of sodium hydroxide to pass and the Kjeldahl bulb the least. Much less nonvolatile alkali passed through the two scrubbing-type bulbs. The experimental bulb was more than three times as effective as the Davisson and more than thirteen times as effective as the best nonscrubbing type. These data indicate that bulbs having only baffles are ineffective, and when used, the conditions of the distillation must be controlled to give a minimum amount of droplets in the vapor stream. Only those bulbs which scrub the vapors will perform satisfactorily independent of the distillation conditions.

Using the Davisson scrubber, the best of the common connecting bulbs, as shown by the preceding experiment, the blank values obtained under ordinary Kjeldahl conditions ranged from a low of 0.2 ml to a high of 0.6 ml of 0.1 *N* hydrochloric acid. The experimental connecting bulb under the same conditions gave a blank value of 0.0 to 0.04 ml of 0.1 *N* hydrochloric acid.

TABLE 1.—*Comparison of various types of Kjeldahl connecting bulbs for removal of entrained nonvolatile alkali*

BULB	DISTILLATION OF 200 ML. PER 30 MIN.			DISTILLATION OF 100 ML PER 30 MIN.
	WITH MOSSY ZINC	WITH 20-MESH ZINC	WITHOUT ZINC	WITH MOSSY ZINC
Scrubber Type				
Experimental bulb	1.82* 1.89 Ave. 1.9	1.30 1.12 Ave. 1.2	1.33 1.06 Ave. 1.2	1.40* 1.68 Ave. 1.5
Davisson bulb	5.69 6.45 Ave. 6.1	3.00* 2.60 Ave. 2.8	1.57* 1.72 Ave. 1.7	2.79 3.67 Ave. 3.2
Nonscrubber Type				
Kjeldahl bulb	23.02 26.92 Ave. 24.9	2.16 1.36 Ave. 1.8		19.4 24.0 Ave. 21.7
Iowa State bulb	56.96 69.93 Ave. 63.5	91.14 36.89 Ave. 64	1.67 1.83 Ave. 1.8	77.0 61.0 Ave. 69.0
Hopkins bulb	152.0 161.0 Ave. 156	164.0 187.0 Ave. 176		64.0 210.0 Ave. 137

* Amount of nonvolatile alkali carried to the receiver expressed as milliliters of 0 01 *N* HCl

An attempt was made to evaluate the two parts of the new bulb, the screen plates and the bulb trap, but it was found that the values obtained by independent measurements of each part were of little significance and that the bulb could be considered only as a whole. The bulb was especially useful as an antifoam trap. In this respect it was far superior to the other bulbs tested.

The rate at which ammonia was distilled into the receiver was determined by preparing several Kjeldahl flasks, all containing the same amounts of reagents and sample. After digestion, alkali was added, and the ammonia distilled into boric acid. The rate of distillation, 200 ml per 30 minutes, was the same for all flasks. The receiving flasks were then withdrawn at different time intervals, beginning at 7½ minutes from the start of boiling. The results are given in Table 2. At the rate of 200 ml per 30 minutes, essentially all the nitrogen as ammonia was distilled during the first 22.5 minutes, and a prolonged distillation did not yield appreciably higher values.

TABLE 2.—*Rate of distillation of ammonia from Kjeldahl digestion mixtures*

DISTILLATION TIME (MIN.)	VOLUME DISTILLED ML. (APPROX.)	NITROGEN FOUND, PER CENT	PER CENT OF TOTAL (THEORY) NITROGEN DISTILLED
7.5	50	7.96 7.93 Ave. 7.94	95.9
15.0	100	8.19 8.12 Ave. 8.16	98.6
22.5	150	8.25 8.26 Ave. 8.26	99.8
30.0	200	8.26 8.31 Ave. 8.29	100.1
37.5	250	8.25 8.23 Ave. 8.24	99.5

Tests were also made to determine whether any ammonia was lost during the initial heating period by being carried through the trapping liquid in the large bubbles of the displaced air. Using distilled water and indicator, we demonstrated that no ammonia was carried by the displaced air, since the indicator did not change color until condensed water vapor had reached the receiver. Loss of ammonia by this cause is therefore improbable.

Although the new bulb made it possible to measure accurately the nitrogen blank of the reagents, it did not account for the slightly low nitrogen values obtained on analysis of pure nitrogenous compounds. This loss could be accounted for only by assuming that all the ammonia was not being caught by the trapping liquid. In an attempt to eliminate this source of error, a Goessman trap (2) was used on the receiver. Results of analyses of S-benzyl thiuronium chloride with the new connecting bulb, with and without the Goessman trap, are given in Table 3. The Goessman trap caused an increase of 0.5 per cent of the total nitrogen found when boric acid was the trapping liquid and 0.7 per cent when 0.1 N hydrochloric acid was used. Results most closely approximating the theoretical were obtained when boric acid was used.

Of the many mixed indicators proposed in the past few years, a mixture of methyl red and methylene blue is often used in Kjeldahl nitrogen titrations. This indicator has been used in many proportions of methyl red to methylene blue, but 4 parts of methyl red to 1 part of methylene blue, with a final concentration of methyl red of 0.1 per cent wt./vol. in 95% alcohol was the most satisfactory with boric acid. Under these conditions ammonium hydroxide is titrated with the mineral acid, usually hydrochloric. The grey, neutral color of this mixed indicator coincides exactly with the stoichiometric end point (pH 4.7). A 4% boric acid solution, however, is acid to the above mixed indicator, and a correction must be made for this in the determination. By the use of the proposed connecting bulb, or by observing the necessary precautions with reagents suitable for Kjeldahl nitrogen analysis, the alkali distilled in the blank is not sufficient to neutralize the boric acid. The acidity of the boric acid of the blank may be neutralized by back titration with a standard base, but a standard base tends to nullify most of the advantages of boric acid as the trapping liquid. The standard base can be omitted if the color of the indicator in the partially neutralized boric acid of the blank is taken as the end-point color. It will be found that this slightly acid shade of the indicator

TABLE 3.—*Nitrogen analysis of S-benzyl thiuronium chloride* by the Kjeldahl method with a new connecting bulb*

LIQUID IN RECEIVER	WITH GOSSMAN TRAP		WITHOUT TRAP	
	NITROGEN, %	RECOVERY, %	NITROGEN, %	RECOVERY, %
Boric acid	13.80		13.82	
	13.78		13.81	
	13.84		13.70	
	13.81		13.78	
	13.81		13.76	
	13.80		13.70	
	Ave. 13.81	99.9	Ave. 13.76	99.6
HCl 0.1 N	13.73		13.63	
	13.72		13.66	
	13.70		13.56	
	13.76		13.60	
	13.75		13.64	
	13.67		13.57	
	Ave. 13.72	99.3	Ave. 13.61	98.5

* % Nitrogen found by the Dumas method equals 13.82.

(*p*H 4.2) is easily matched, and in the analysis of a sample the titration will be carried through indicator color changes from green to grey to light purple (end point shade). The grey shade serves as a warning of the approach to the end point. By taking the sample titration to the same indicator color as that developed in the blank, no blank correction need be used in the calculation, since this procedure automatically corrects for the reagent nitrogen and the boric acid acidity.

SUMMARY

Some of the sources of errors which occur during distillation in the Kjeldahl method for determination of nitrogen have been pointed out. Chief among these is the carry-over of nonvolatile alkali from the alkaline distilling mixture to the trapping liquid in the receiver. By the use of a new connecting bulb, the carry-over of non-volatile alkali is greatly reduced, practically eliminating this source of error. A titration technique is proposed with the mixed indicator, methyl red-methylene blue, which eliminates the standard base and simultaneously corrects for any base from the reagents as well as for the acidity of the boric acid.

LITERATURE CITED

(1) Davisson, B. S., *Ind. Eng. Chem.*, **11**, 465 (1919).
(2) Goessman, C. I., Mass. Agric. Expt. Sta. Bulletin 54 (1898).
(3) Hopkins, C. G., *J. Am. Chem. Soc.*, **18**, 227 (1896).
(4) Lovecy, A., *J. Soc. Chem. Ind.*, **65**, 224 (1946).

ADDITION OF SELENIUM TO WET-ASH PROCEDURE FOR THE DETERMINATION OF MERCURY IN APPLE PEEL*

By Frieda M. Kunze (Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.)

The method reported by Laug and Nelson[1] for the determination of mercury in biological material was investigated for its adaptability to the mercury analyses of apples sprayed with phenyl mercuric ammonium lactate. It was found that the recovery of mercury added to animal diet, tissues, or excreta in an order of 0.2 p.p.m. could be effected with ease and accuracy, but that the recovery of much larger quantities of mercury added to apple peel or pulp was uniformly poor. Complete recovery of mercury could be obtained, however, if the mercury was added to the acid apple digest subsequent to the wet-ashing procedure, indicating that in the instances of low recoveries the mercury was either complexed during the oxidation procedure or that it was lost through volatilization. Analysis of the acid contained in a trap fitted to a Friedrichs extraction condenser showed that no loss of mercury occurred by refluxing if the oxidizing mixture was added cautiously during the violent frothing period. To minimize the diluting effect, wet apple peel was dried overnight on a flat plate in an oven under forced draft at 50°C. This step increased the efficacy of the oxidizing mixture, but appreciable loss of mercury occurred by volatilization during the drying.

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[1] Laug, E. P., and Nelson, K. W., *This Journal*, **XXV**, 400 (1942).

An adaptation of Hubbard's method[2,3] essentially a sulfuric-nitric digestion followed by $KMnO_4$, was rejected because of a variable mercury blank due, no doubt, to impure $KMnO_4$. Cognizance of the use of selenium as a catalyst in nitrogen determinations and of the fixative properties of mercuric oxide in selenium determinations[4] prompted an investigation of its use here. Selenium was added either as the metallic powder or as an aqueous solution of sodium selenite. Both effected quantitative recovery of mercury from apple peel.

PREPARATION OF SAMPLE

The modifications in the ashing method of Laug and Nelson are detailed below. In every other respect the procedure is unchanged.

REAGENTS

(1) *Metallic selenium or sodium selenite, reagent grade.*

(2) *NaOH, 50% W/V, a.c.s.*—Prepare a stock supply and store in a paraffin-lined bottle.

(3) *Superoxol.*—Hydrogen Peroxide, 30%, c.p.

(4) All other reagents listed by Laug and Nelson.

Transfer 50 grams wet apple peel to the distilling flasks; add *ca.* 0.1 gram metallic selenium or an equivalent amount of sodium selenite and shake until the selenium is well equalized throughout the sample. Over an interval of 5–10 min., add cautiously, through the drip funnel, 15 ml of concentrated sulfuric acid and 5 ml of concentrated nitric acid. (An initial 3:1 sulfuric nitric ratio reduces frothing and decreases the reflux time. Considerable variation will be found in the amount of acid necessary to complete digestion. Usually 50 grams of wet apple peel require 20 ml of sulfuric and 30–40 ml of nitric acid.) Whenever charring occurs add small amount of nitric acid. As the frothing abates, increase the heat and reflux at full flame for about 2½ hours or until the soln is clear; cool; add 10 ml H_2O_2; reflux for another half hour. Since relatively large amounts of acid and small amounts of mercury are involved and in order to prevent oxidation of dithizone during the extraction procedure, neutralize the acid digest with 50% NaOH to a *p*H of 1 to 1.5, or use an aliquot containing no more than 2.5 ml of H_2SO_4. (Over-neutralization will result in mercury loss.) Proceed with the determination of Hg as outlined by Laug and Nelson.[1]

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[2] Cholak, J., and Hubbard, D., *Ind. Eng. Chem., Anal. Ed.*, 12, 768–771 (1940); Hubbard, D., *Ind. Eng. Chem., Anal. Ed.*, 18, 149 (1946).

[3] Unpublished experiments. Food Division, Food and Drug Administration, Federal Security Agency.

[4] Klein, A. K., *This Journal*, XXVI, 347 (1943).

TABLE 1.—*Mercury recovered from apple peel*

1:1 SULFURIC NITRIC			1:1 SULFURIC NITRIC+Se			SULFURIC NITRIC+KMnO$_4$			
Hg ADDED	Hg RECOVERED	PER CENT RECOVERED	Hg ADDED	Hg RECOVERED	PER CENT RECOVERED	Hg ADDED	Hg RECOVERED	BLANK	PER CENT RECOVERED
mmg	*mmg*	*mmg*	*mmg*	*mmg*	*mmg*	*mmg*	*mmg*	*mmg*	*mmg*
				Dry peel—8 grams					
69.0	21.7	36	20	20.3[a]	101				
55.0	30.8	56	60	60.3[a]	100				
75	30.0	40	60	62.4[a]	104				
43	22.5	52							
				Wet peel—50 grams					
			20.0	20.4[a]	102	8.0	8.0	1.41	83
			70.0	62.6[b]	89	8.0	8.76	1.58	90
			30.0	30.4[b]	101	9.0	9.75	1.75	89
			2.0	1.76[b]	88				
			5.0	4.86[b]	97				
			4.0	4.20[b]	105				

[a] Unneutralized aliquot used.
[b] Partially neutralized aliquot.

Acid digestion will not destroy completely the wax present in the sample. Filter through glass wool and make up to suitable volume (100–200 ml). The sample or aliquot to be analyzed must be cooled before extraction to prevent precipitation of Se by $NH_2OH \cdot HCl$. A rust-red color which sometimes appears in the stem of the funnel, however, does not interfere with mercury determination.

DISCUSSION

The neutralization procedure introduces a small blank. After the insoluble matter in the NaOH solution had settled, however, this blank became constant. For instance, 50 ml of 50% sodium hydroxide contributed 0.85 microgram of mercury.

There is evidence that selenium exerts a fixative action on mercury; therefore, a study was undertaken to determine the feasibility of a Kjeldahl digestion procedure. Under carefully controlled conditions fairly good recoveries were obtained from apple peel, but the same procedure applied to rat diet and beef liver yielded low results. The time and care required and the questionable reproducibility invalidates the open Kjeldahl digestion.

RESULTS

Table 1 shows the recoveries obtained when the apple peel was ashed by three different methods. The addition of selenium to sulfuric-nitric oxidizing mixtures effected quantitative recovery without contributing to the blank. Neutralization of the ash with 50% NaOH adds an analytical blank.

The author wishes to thank Edwin P. Laug, Alfred K. Klein, and Hugo J. Wichmann, Food and Drug Administration, for their suggestions and comments.

SEPARATION OF THE SATURATED STRAIGHT-CHAIN FATTY ACIDS C_{11} TO C_{19}*

By L. L. RAMSEY and W. I. PATTERSON (Food Division,† Food and Drug Administration, Federal Security Agency, Washington 25, D. C.)

For analysis of fats, the fatty acids are usually separated by the fractional distillation of their methyl or ethyl esters, and apparatus is available for separating less than a gram of the mixed esters (1). Low temperature crystallization is also being used more frequently for fatty acid purification and separation, but this technique is not readily adapted to micro quantities. Both distillation and crystallization are either time consuming or else require special apparatus. In a search for a more reliable and simpler procedure for small quantities of fatty acids the technique termed "partition chromatography" (2) was investigated. The relatively easy quantitative separation of the fatty acids with less than 11 carbon atoms by partition chromatography (3) suggested that the same principle might apply to the higher fatty acids. Earlier use of adsorption chromatography for complete separation of these fatty acids was not too promising, although the separation of the higher acids, including palmitic from myristic, was achieved (4).

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† W. B. White, Chief.

Whereas water was used as the immobile solvent for the lower acids (5), the low solubility of the higher acids in water precluded its use in a partition system for their separation. Nitromethane had already been used as the immobile solvent, with n-hexane as the mobile solvent, for the separation of some isomers of hexachlorocyclohexane (6), but these solvents were not suitable for separating the higher fatty acids. Methanol and 2,2,4-trimethylpentane were an acceptable combination for the acids through capric (3), but again were unsuitable for the higher acids.

In the further search for a suitable pair of immiscible solvents for separating the higher fatty acids, preliminary experiments indicated that furfural, furfuryl alcohol, tetrahydrofurfuryl alcohol, or β-hydroxyethyl pyridine as the immobile solvent, with 2,2,4-trimethylpentane or n-hexane as the mobile solvent, would separate lauric from palmitic acid, but would not separate palmitic from stearic acid completely. Finally, a mixture of furfuryl alcohol and 2-aminopyridine as the immobile solvent, with n-hexane as the other solvent, gave a satisfactory separation of palmitic and stearic acids, and a mixture of the even-numbered acids C_{12}–C_{18}.

The synthetic acids containing an odd number of carbon atoms from C_{11} through C_{19} were also tested on this column and found to separate in a manner similar to that of the even-numbered acids. When the C_{18} acid (stearic) was mixed with the C_{19} acid (nonadecanoic) in equal amount, separation was only partial; however, the first portion of the percolate containing ca ¼ of the eluted acids was pure C_{19} within the limits of detection. Suitable refractionation of the remaining eluate results in recovery of more of the pure C_{19} acid, and also of part of the C_{18} acid. Thus a single passage through the column is capable of completely separating from each other, within the limits of detection, only the even-numbered members of the series C_{12}–C_{18}, and only the odd-numbered members of the series C_{11}–C_{19}. The purity of the separated acids is rather difficult to establish, since by the usual criteria of purity even 1 per cent of a homolog, present as an impurity in one of these acids, is difficult to detect.

The identification of each acid may be made tentatively by measuring its threshold volume as was described in an earlier paper, for the C_6 to C_{10} acids (3). This tentative identification may be confirmed by a melting point determination or by ascertaining the chromatographic homogeneity of a mixture of the unknown with an approximately equal amount of an authentic sample of the suspected acid.

With the weakly basic 2-aminopyridine as an essential part of this solvent system, an equilibrium of the free fatty acids with their 2-aminopyridine salts is probable, and this may contribute to the success of the method. However, the solvent properties of the 2-aminopyridine also seem to be an important factor, since experiments with a series of aqueous buffers as the immobile phase, and n-hexane as the mobile one, gave no separation at all. Attempts to determine whether it is the free acids or their 2-aminopyridine salts that are actually separated led to inconclusive results.

Whereas bromocresol green was used as an indicator for the C_6 to C_{10} acids, in the presence of the basic 2-aminopyridine an indicator will not change color with the relatively minor amounts of acid going through. Even in the separation of lauric from palmitic acid with no 2-aminopyridine present, the indicators previously used (3, 5) showed no appreciable change in color in the presence of small quantities of the acids above lauric. Thus the separation of these higher acids, as now carried out, is followed by the collection of a series of percolate fractions and the measured titration of each.

The purity of the solvents, 2-aminopyridine and furfuryl alcohol, does not seem to affect the separation of the fatty acids, as technical and purified material gave the same threshold volume, recovery, and other behavior.

With columns of the size employed, the optimum working range of the method is from about 5 mg to 20 mg of each acid, the lower limit being defined by the sensitivity of the titration rather than by the sensitivity of the separation.

The saturated straight-chain fatty acids below hendecanoic (C_{11}) do not interfere with the separation of the C_{11}–C_{19} straight-chain members of the series. The naturally-occurring unsaturated fatty acids, such as oleic, do interfere and must be removed by suitable means before the method can be applied to the acids of a hydrolyzed fat.

METHOD

APPARATUS

(1) Chromatographic tubes.

(a) Tube 27 mm O. D. × 200 mm long prepared from Pyrex tubing, or from Pyrex test tubes by sealing into the bottom of each a piece of glass tubing 6 mm O. D. × 25 mm long.

(b) Any other suitable container.

(2) Suitable pressure source, such as compressed air or a cylinder of nitrogen; and a means of keeping the pressure constant, such as a column of mercury or a diaphragm type pressure regulator.

REAGENTS

(1) *Silicic acid.*[1]

(2) *Furfuryl alcohol*[2]—*2-aminopyridine solvent:*—Dissolve sufficient sodium hydroxide in furfuryl alcohol to make it strongly alkaline, and distil. Dissolve 50 g of 2-aminopyridine in 50 g of purified furfuryl alcohol.

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[1] Mallinckrodt's S.L. grade precipitated powder was used in this work

[2] Purification of these solvents is included in order to remove any acidic materials which might be present.

(3) *n-Hexane*[2] *solvent:*—If not free of acid, wash with alkali, dry, and distil. To ca. one l of n-hexane add 25 ml of the furfuryl alcohol-aminopyridine solvent, shake vigorously, allow to separate, draw off the furfuryl alcohol-aminopyridine layer, and reserve it for saturating a future batch of n-hexane.

(4) *Phenolphthalein soln:*—Dissolve 1 g of phenolphthalein in 100 ml of absolute alcohol and neutralize with sodium ethylate.

(5) *Sodium ethylate, standard 0.02 N soln.*—Prepare this soln by the usual procedure using aldehyde-free absolute alcohol.

(6) *Sodium hydroxide, standard 0.02 N soln.*

(7) *Ethyl alcohol,* 70% v/v:—Dilute 700 ml of 95% alcohol to 950 ml. Neutralize just before use with the 0.02 *N* NaOH.

(8) *Ether, A.C.S.*

(9) *Sulfuric acid,* ca *N.*

(10) *Hendecanoic, lauric, tridecanoic, myristic, pentadecanoic, palmitic, margaric, stearic, and nonadecanoic acids.*

PROCEDURE

(1) *Preparation of the chromatographic column and determination of the optimum ratio of furfuryl alcohol-aminopyridine solvent to the silicic acid:*

To 20 g of silicic acid in a mortar, add 10 ml of the furfuryl alcohol-aminopyridine solvent and mix well with a pestle. Add ca 65 ml of the n-hexane solvent and make a slurry. Place a very small cotton plug firmly in the neck of the constriction at the bottom of the chromatographic tube and clamp the tube in an upright position. Pour the slurry into the tube, preferably by transferring from the mortar to a 100 ml beaker and pouring from the beaker into the tube; connect the top of the tube to a suitable pressure source thru the gas pressure regulator; and apply 2–5 pounds of pressure. During the packing down process, tap the tube from time to time to aid in obtaining a smooth, level surface. Observe whether the gel packs down quite firmly without cracking or drying out, and whether the flow rate is convenient—in the range 3–5 ml per minute. If the flow rate greatly exceeds 5 ml per minute and/or the gel fails to pack down firmly without cracking or drying out, it is possible that too much furfuryl alcohol-aminopyridine (immobile) solvent has been used. On the other hand if the flow rate is extremely slow (less than 2 ml per minute), the silicic acid probably has a greater capacity for the immobile solvent. Accordingly, increase or decrease by ca 2 ml the amount of the immobile solvent to be added to another 20 g portion of silicic acid, and prepare another column as above. Observe its behavior. Proceed in this manner until the optimum ratio of furfuryl alcohol-aminopyridine solvent to silicic acid (*i.e.*, the ratio which produces a column that packs down firmly without cracking or drying out, and with a convenient flow rate) has been found. If a column prepared with the optimum ratio of immobile solvent to silicic acid begins to crack or dry out because the pressure was left on too long in the packing down process, remove the gel from the tube, slurry again with the hexane, retransfer to the tube, and repack.

(2) *Testing the silicic acid for its suitability and standardization of the chromatographic column:*

Pipet onto a column, prepared as in (1) above, 2 ml of a standard solution of lauric, myristic, palmitic, and stearic acids[3] in hexane containing about 10 mg of each acid. Place a graduated cylinder under the column to collect the percolate, and allow the solvent to sink into the gel under pressure. At the instant all the solution has sunk into the gel, release the pressure, and add 1 ml of hexane. Renew the pressure until this wash solvent has sunk into the gel, and repeat the washing again using 1 ml of hexane. Fill up the tube with solvent and renew the pressure. (A separatory funnel or other suitable container may be fitted into the chromatographic tube to serve as a reservoir for the solvent.)

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[3] Since the odd- and even-numbered carbon acids are ordinarily not found together, and since a single passage through the column will not completely separate a mixture of contiguous fatty acids, the standardization is made with a mixture of the acids with either an even or an odd number of carbon atoms. For brevity the procedure is given only for the even-numbered acids.

After 30 ml of solvent has percolated through the column, begin to collect 2 ml fractions in a 5 ml graduated cylinder. When the first 2 ml fraction has been collected, transfer it immediately to an Erlenmeyer flask and titrate with the 0.02 *N* sodium ethylate using phenolphthalein as the indicator. Add the second fraction to the first in the Erlenmeyer flask and again titrate; continue adding successive fractions to this flask, titrating each time, until a titration is obtained greater than the preceding one by several hundredths of a ml. The cumulative volume just before this fraction is the threshold volume for stearic acid. Continue titrating and recording both the cumulative volumes and titrations, until the volume of alkali decreases to ca 0.1 ml for a 2 ml fraction. Now transfer each 2 ml fraction to flask 2 until only 0.05 ml or less of alkali is required. (Another flask is used in order to obtain sensitivity of titration for these small amounts of acid.) Combine flasks 1 and 2 and label "stearic acid."

At this stage begin collecting, in flask 3, 5 ml fractions and titrate each as before; continue to titrate each fraction until only ca 0.05 ml is required. Transfer the next fraction to flask 4 and continue as before until only ca 0.02 ml of alkali is required. Combine the percolates in flask 3 and 4 and label "palmitic acid."

Continue collecting and titrating 5 ml portions of percolate as for palmitic acid, first in flask 5 and then in flask 6. Combine the material in flasks 5 and 6 and label "myristic acid." At this point collect, in flask 7, 10 ml portions until the titration becomes 0.02 ml or less. Label flask 7 "lauric acid."

From the record of volumes collected ascertain the threshold volume for each of the other three fatty acids. These threshold volumes constitute the standardization of the column.

Using the above procedure, run through the process, omitting the addition of the acids to the column, in order to obtain the blank. Correct the titrations of the 4 acids for the blank and calculate the approximate amount of each acid recovered based on the titration.[4]

For more accurate results, distil off the solvent from the sodium salts of the four acids, dissolve each of the salts in ca 20 ml of water, and transfer to individual separatory funnels with a small amount of water. Acidify each of the solutions with 2 ml of the ca *N* H_2SO_4 and extract successively with 25 and 10 ml portions of ether, drawing off and combining the ether extracts for each acid in another separatory funnel. Wash each of the combined ether extracts once with ca 5 ml of water and pour the ether solution through a small folded filter into a 125 ml Erlenmeyer flask. Wash the funnel and filter paper with three 10 ml portions of ether. Evaporate the ether on the steam bath, dissolve the acid in neutral 70% alcohol, using heat if necessary to effect solution, and titrate with the standard 0.02 *N* NaOH, using phenolphthalein as indicator. From the titration in each case, calculate the amount of acid present.

Consider the silicic acid suitable if, upon following the above procedure, the recoveries of the added acids are in the range 90–100%.

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[4] The titration of a fatty acid in hexane in the presence of 2-aminopyridine, using phenolphthalein as the indicator, is almost quantitative, the results being only a trifle high; but the total titration of a series of cumulative fractions as required by the method leads to high and somewhat non-reproducible results even when the titrations are corrected for a blank.

(3) *Determination and identification:*

Determine the acids in an unknown exactly as described above in the standardization and suitability procedure.

Compare the threshold volumes of the unknown acids with those of the known acids and make a tentative identification of the acids.

To confirm the tentative identification proceed as follows: (a) Where at least 10 mg of the acid is available, crystallize the acid from hexane in a 15 ml centrifuge tube (or small test tube) by cooling to ca minus 10°C. Isolate the acid either by centrifuging at that temperature, washing, and drying in the centrifuge tube; or by filtering with suction at ca minus 10°C. on a small filter. Determine the melting point. (b) With the smaller amounts proceed as follows: Pipet 1 ml of a hexane solution containing 5 to 10 mg of the unknown acid onto a freshly prepared column. Pipet an approximately equal amount of the authentic acid dissolved in ca 1 ml of hexane onto the column; gently swirl to mix; and proceed to develop the chromatogram. If only one band is obtained, *i.e.*, if all the acid elutes in the same volume of solvent required in the standardization process to elute the authentic acid, the tentative identification is confirmed.

EXPERIMENTAL RESULTS AND DISCUSSION

Table 1 shows the recoveries of the fatty acids with an even number of carbon atoms when present in admixture in the range 3 to 20 mg. each. Two different mixtures were analyzed, one of them in duplicate. As can be seen, recoveries are generally within 6 or 7 per cent. No corrections

TABLE 1.—*Recoveries and threshold volumes of lauric, myristic, palmitic, and stearic acids when present in admixture*

ACID	ADDED	FOUND	RECOVERY	THRESHOLD VOLUME
name	*mg*	*mg*	*per cent*	*ml*
		Mixture A		
Stearic	10	10.0	100	68
		9.3	93	66
Palmitic	10	9.7	97	106
		9.8	98	102
Myristic	10	9.7	97	170
		9.7	97	165
Lauric	10	9.4	94	275
		10.0	100	280
		Mixture B		
Stearic	10	9.3	93	68
Palmitic	20	18.8	94	102
Myristic	5	5.5	110	175
Lauric	3	3.1	103	295

have been made for homologs found (as later described) as impurities in the acids used, but in no case would the results in this table be appreciably affected by such impurities. Table 1 also shows the threshold volumes obtained in each experiment for each acid. The threshold volume of an acid is not appreciably affected by the quantity of acid, within the working range of the method.

Table 2 shows the recoveries of the fatty acids with an odd number of carbon atoms when such acids are present in admixture. As can be readily seen from the data the recoveries are of about the same degree of precision as those of the even-numbered acids.

TABLE 2.—*Recoveries and threshold volumes of hendecanoic, tridecanoic, pentadecanoic, margaric, and nonadecanoic acids when present in admixture*

ACID	ADDED	FOUND	RECOVERY	THRESHOLD VOLUME
name	*mg*	*mg*	*per cent*	*ml*
Mixture A				
Nonadecanoic	10	9.4	94	54
Margaric	10	9.4	94	80
Pentadecanoic	10	10.0	100	121
Tridecanoic	10	9.5	95	220
Hendecanoic	10	9.3	93	370
Mixture B				
Nonadecanoic	20	18.8	94	54
Margaric	20	19.7	98	82
Pentadecanoic	20	19.4	97	121
Tridecanoic	20	19.1	95	215
Hendecanoic	20	18.9	94	360

The melting points of the separated acids (Table 3) were determined on a Fisher-Johns melting point apparatus. A more precise melting point was unnecessary to distinguish between adjacent acids with an even number of carbon atoms, or between adjacent acids with an odd number of carbon atoms, after each had been separated from its homologs by passage through the column and recrystallized once.

An experiment was performed to determine the probable degree of contamination of the even-numbered acids with their even-numbered homologs after separation by the proposed method. Refractionation of the separated acids was not feasible because of the difficulty of measuring the small amounts of acid involved. For each of the four even-numbered acids, the threshold volume and the volume of eluate required to elute 98–100 per cent of each acid (10 mg. quantity) were determined on the same

TABLE 3.—*Melting points of the acids after chromatographic separation of admixtures of 20 mg each followed by recrystallization from hexane, compared with melting points from literature* (7)

ACID	M.P., ° C. (7)	M.P., ° C. FOUND
Admixture of acids containing an even number of carbon atoms		
Stearic	69.6	68–69
Palmitic	63.1	61–63
Myristic	53.9	52–53
Lauric	44.2	41–42
Admixture of acids containing an odd number of carbon atoms		
Nonadecanoic	68.6	67–68
Margaric	61.3	59–61
Pentadecyclic	52.3	51–52
Tridecylic	41.5	40–41
Hendecanoic	29.3	23–26*

* Satisfactory recrystallization of this acid (20 mg) was difficult.

column, a single acid being placed on the column and then completely eluted before the next was added. After all four acids had been passed through the column, the threshold volume for stearic acid was checked to determine whether the column had changed; this threshold volume was found to remain the same. The results are tabulated in Table 4. Based on the data in Table 4 it appears that the separations are at least 98 per cent complete in all cases, and probably better than that in the case of lauric and myristic acid and in the case of myristic and palmitic acid.

TABLE 4.—*Fractions containing the even-numbered fatty acids*

ACID	FRACTION CONTAINING 98–100% OF THE ACID*
Name	*ml*
Stearic	68–98
Palmitic	100–145
Myristic	160–225
Lauric	275–390

* First figure of a fraction is the threshold volume.

An experiment was conducted to determine the sensitivity of the method in detecting the even-numbered homologs of an even-numbered acid added to such acid as impurities. Using a 40 mg. sample and a single fractionation, 5 per cent of added homolog could be detected; with a larger sample and suitable refractionation, about 0.5 per cent could be detected.

The even-numbered fatty acids used in this work were examined by the proposed method to determine their purity. The results are tabulated in Table 5. A weighed quantity of the fatty acid in the range 200 to 300 mg. was dissolved in 5 to 10 ml of hexane and transferred to the standard 20 g column. The fraction beginning after about 50 ml of solvent had passed through the column and including a small part of the main band (5 to 10%) was collected and designated as fraction 1. (This fraction was not collected for stearic acid.) The middle portion of the main band was collected as fraction 2 and discarded. Beginning 5 to 10 ml before the next lower homolog should begin to elute, fraction 3 was collected and collection was continued until no more acid would elute. Fraction 1 containing a small amount of the main band and the higher homologs, if any, was refractionated on a fresh column. The higher homologs, if any, were identified by threshold volume and determined by titration. A third or fourth refractionation was made when necessary. Fraction 3 containing the tailings of the acid in the main band plus its lower homologs, if any, was refractionated on a fresh column and the fractions identified and measured in a manner similar to that for fraction 1.

TABLE 5.—*Method applied to the even-numbered acids used in this work to determine their purity*

ACID	GRADE	HOMOLOGS FOUND AS IMPURITIES
Stearic	Pure	2% palmitic acid
Palmitic	Pure	0.5% stearic acid
Myristic	Pure	0.5% palmitic acid and 0.8% lauric acid
Lauric	Pure	1.5% capric acid

The purity of the odd-numbered acids (obtained from commercial sources) used in this work was also investigated by the same general procedure used above for the even-numbered acids. The margaric acid was found to contain about 30 per cent of non-acidic material and 17 per cent of nonadecanoic acid. Tridecanoic acid was found to contain 12 per cent of lauric acid and 7 per cent of hendecanoic acid. Each of these acids was purified chromatographically for the recovery experiments reported above, approximately 200 mg of each being prepared. For this purification a standard 20 g column was used. In the case of margaric acid, about 150 mg of the acid dissolved in ca 10 ml of hexane was fractionated on the column. The fraction 88–110 ml containing 35 to 40 mg of acid was collected, the forerun and tailings being discarded. The same column was usually used in succession for three 150 mg batches of the acid. A total of six fractions from six 150 mg batches of the acid were combined; the hexane solution was washed once with approximately 0.1 *N* sulfuric acid and 3 or 4 times with water to remove the 2-aminopyridine. The solution was then dried over anhydrous sodium sulfate, filtered, and evaporated on the steam bath until the concentration was about 5 mg per ml, the concentration being accurately determined on an aliquot by titration. A refractionation of 50 mg of this purified acid (solution concentrated to about 3 ml before addition to the column) showed that no nonadecanoic acid was present. Since the nonadecanoic acid is almost completely eluted before the margaric acid begins to come through near the 80 ml point, beginning the collection of the "pure" fraction at 88 ml in the purification process above gives about an 8 ml fraction as a margin of safety.

In the case of tridecanoic acid ca 100 mg of the acid dissolved in about 5 ml of hexane was placed on a standard 20 g column and the fraction 205–275 ml (containing ca 60 mg acid) collected, the forerun and tailings being discarded. The fractionation of each 100 mg batch of acid was made on a fresh column, a total of 4 columns being used to produce about 200 mg of tridecanoic acid free of lauric acid. The hexane solution of the tridecanoic acid was washed, dried, filtered, and concentrated as above for margaric acid. A refractionation of 50 mg of the purified acid indicated that not more than a trace of lauric acid was present; 98 per cent of the acid was eluted in the fraction 215–280 ml.

TABLE 6.—*Threshold volumes of the even-numbered fatty acids with different mobile solvents and with 2-aminopyridine-furfuryl alcohol as the immobile solvent*

ACID	MOBILE SOLVENT, ML		
	HEXANE	CYCLOHEXANE	2,2,4-TRIMETHYLPENTANE
Stearic	68	52	102
Palmitic	106	80	170
Myristic	170	128	295
Lauric	275	220	530

The nonadecanoic acid contained no homologous acids, but did have ca 40 per cent of non-acidic impurities. This acid was also purified chromatographically before use in a procedure similar to the above.

Certain other solvent combinations in which the threshold volume of stearic acid is higher may be useful for separating still higher fatty acids, assuming that their threshold volumes will be less than those of the acids with fewer carbon atoms. As the threshold volume increases, the width of the band containing a given acid also increases; thus the completeness of separation of two acids may be just as good with relatively low threshold volumes as it is with higher ones. Table 6 affords a comparison of the threshold volumes of the even-numbered acids C_{12}–C_{18} using three different mobile solvents with the same immobile solvent. It was also found that the separations with cyclohexane were of about the same order of completeness as with hexane or 2,2,4-trimethylpentane.

It was found that the threshold volumes for palmitic acid shown in

Table 6 are in line with its distribution coefficients in the various solvent systems, one member of which was always a mixture of 2-aminopyridine and furfuryl alcohol. The distribution coefficients were determined as follows:

> The 2-aminopyridine-furfuryl alcohol solvent was prepared by dissolving sufficient 2-aminopyridine in furfuryl alcohol to double the volume of the furfuryl alcohol taken. The cyclohexane and 2,2,4-trimethylpentane were pure grade reagents and were used directly without redistilling. The solvents were saturated with each other and 25 ml of each solvent were pipetted into a separatory funnel. One hundred mg of palmitic acid were added and the funnel shaken vigorously for several minutes. After the phases separated the lower layer was drained off and discarded. A 20 ml aliquot of the upper, or hydrocarbon, layer was pipetted into a beaker and the hydrocarbon boiled off on a steam bath. The residue was dissolved in neutral 70% alcohol and titrated with 0.02 *N* NaOH using phenolphthalein as the indicator. From the titration the amount of palmitic acid present in the hydrocarbon layer was calculated, and the amount of acid present in the 2-aminopyridine-furfuryl alcohol layer obtained by difference. The distribution coefficient in the results below is defined as the ratio of the quantity of palmitic acid in the 2-aminopyridine-furfuryl alcohol to the quantity of the acid in the hydrocarbon. For the solvent system with hexane the distribution coefficient of palmitic acid was found to be 5.6; with cyclohexane, 3.7; and with 2,2,4-trimethylpentane, 8.3.

The threshold volume of a fatty acid is also affected by the particular silicic acid used, probably by its adsorptive capacity, which varies somewhat from one batch of silicic acid to another. For example, with one batch of silicic prepared in the laboratory according to a recommended procedure, (8) the threshold volume of stearic acid on a standard column was 96 ml and that of palmitic acid was 150 ml. (See Table 1 above for the threshold volumes of these acids using a commercial silicic acid as the column medium.)

2-AMINOPYRIDINE STEARATE

When stoichiometric amounts of 2-aminopyridine and stearic acid were dissolved in each of three solvents, alcohol, ether, and furfuryl alcohol, and then set aside to crystallize, the crystals obtained in each case were found to be stearic acid. When stearic acid was mixed with a large excess of 2-aminopyridine in alcohol (2 parts of aminopyridine to 1 part of alcohol, by weight), somewhat impure 2-aminopyridine stearate precipitated. The crude product was purified by recrystallization from n-hexane. However, 2-aminopyridine stearate is best prepared by dissolving stoichiometric amounts of the acid and base in hot hexane and allowing the salt to crystallize. The detailed preparation follows:

> Stearic acid, 2.85 g, and 2-aminopyridine, 0.94 g, were dissolved in ca 90 ml of hexane by heating on the steam bath. A few mg of decolorizing carbon were added and the solution gently boiled ca 5 minutes. The solution was filtered, reheated on the steam bath, and set aside to crystallize at room temperature. The fine needle-like crystals were filtered on a Büchner funnel, washed with two small portions of hexane, and dried. The neutral equivalent of the salt was determined by dissolv-

ing 378.6 mg in 70% alcohol and titrating against 0.1 *N* NaOH using phenolphthalein as the indicator. The neutral equivalent was found to be 377; theory is 378.6. The product melted at 62–64°C.

The salt, 2-aminopyridine stearate, can be separated into its component parts by dissolving it in hexane and washing the hexane solution 4 or 5 times with water. The stearic acid remains in the hexane while the 2-aminopyridine goes into the aqueous phase. One millimol of the 2-aminopyridine stearate (378.6 mg) was dissolved in 50 ml of hexane and the solution washed with four 25 ml portions of water. The wash water portions were combined and titrated against 0.1 *N* HCl using bromocresol green as the indicator; 9.5 ml of 0.1 *N* HCl were required, whereas 10 ml is the calculated amount.

SUMMARY

A method, based on partition chromatography, is presented for the separation of the straight-chain saturated fatty acids C_{11}–C_{19}. The separation of the even-numbered acids from each other and of the odd-numbered members from each other is fairly complete in a single fractionation and recoveries of added acids are essentially quantitative.

The fatty acids are separated on a column of silicic acid using a mixture of furfuryl alcohol and 2-aminopyridine as the immobile solvent, and n-hexane as the mobile solvent. The separation is followed by titration of percolate fractions of suitable volume with standard sodium ethylate, and the separated acids are determined by titration in 70 per cent alcohol with standard sodium hydroxide. Each acid is tentatively identified by its threshold volume, and the identification is confirmed either by a melting point determination or by adding an approximately equal amount of an authentic sample of the suspected acid to the unknown and testing the chromatographic homogeneity of the mixture on a fresh column.

BIBLIOGRAPHY

(1) Craig, L. C., *Ind. Eng. Chem., Anal. Ed.*, **9**, 441 (1937).
(2) Martin, A. J. P., and Synge, R. L. M., *Biochem. J.*, **35**, 1358 (1941).
(3) Ramsey, L. L., and Patterson, W. I., *This Journal*, **31**, 139 (1948).
(4) Kaufmann, H. P., *Fette u Seifen*, **46**, 268 (1939).
(5) Ramsey, L. L., and Patterson, W. I., *This Journal*, **28**, 644 (1945).
(6) ———— *Ibid.*, **29**, 337 (1946).
(7) Markley, K. S., "Fatty Acids, Their Chemistry and Physical Properties," p. 114 (1947).
(8) Gordon, A. H., Martin, H. J. P., and Synge, R. L. M., *Biochem. J.*, **37**, 79 (1943).

BOOK REVIEWS

The Chemistry of Organic Compounds—A Year's Course in Organic Chemistry. By JAMES BRYANT CONANT and ALBERT HAROLD BLATT. Third edition. x+665 pages. The Macmillan Co., 60 Fifth Ave., New York, N. Y., 1947. Price $5.00.

The regulatory chemist's need for a general up-to-date knowledge of the basic principles of organic chemistry is becoming almost a necessity, with the multitude of new synthetic drugs and food adjuncts. Without this elementary knowledge of the recent developments in organic chemistry, it is more difficult for the control chemist to develop or apply analytical procedures suitable for effective enforcement of food and drug laws. On the other hand, the average control chemist does not have time for the details and more involved theories of organic chemistry. An elementary treatment of the subject such as this third edition of "The Chemistry of Organic Compounds" is perhaps a desirable answer. Even though the book is planned as an elementary text, Conant and Blatt have succeeded in describing and illustrating organic chemical principles in a simple, easily readable style which tends to hold the reader's interest.

With examples, accompanied by some discussion, the authors illustrate the significance of certain physico-chemical principles in organic reactions. This recognition of the role which certain thermodynamic principles play in organic reactions is a noteworthy step in the treatment of elementary organic chemistry, as it tends to put previous statement of fact upon a logical and, to some extent, predictable basis.

Industrial products from petroleum, polymerization mechanisms, and the chapter on "Certain Biochemical Processes" are subjects in the book deserving specific mention. Some of the newer antimalarials and other drugs are also described. The few errors which were noted are of the obvious typographical kind. This book is to be recommended to those who desire an up-to-date presentation of organic chemistry.

W. I. PATTERSON

Cottonseed and Cottonseed Products, edited by ALTON E. BAILEY. Interscience Publishers, Inc., New York, 905 pp. Price $17.50.

This book on the chemistry and technology of the cottonseed industry contains chapters written by the editor and twenty-four collaborating authors. While the various chapters are grouped into sections, mention will be made only of the specific chapters. The authors have presented their subject matter in such a well organized manner that there is a minimum of duplication and over-lapping in the various chapters.

The first chapter of 50 pages on the "History of Cotton and the U. S. Cottonseed Industry," by Maurice R. Cooper, gives a historical background and outlines the development of the various phases of the cottonseed industry. Cottonseed processing, production, and consumption in the important cotton-producing nations are discussed in 50 pages by Charles E. Lund. The chapter of 11 pages by John Leahy on the "Structure of the Cottonseed" is enhanced by numerous photographs of the structural characteristics and of the microscopic sections of the cottonseed. In 39 pages, W. H. Thorp covers "Cottonseed Composition—Relation to Variety, Maturity, and Environment of the Plant," which includes numerous tables of data on composition of cottonseed and its different parts. "Biological Processes of the Cottonseed," 55 pages, by Aaron M. Altschul, will be of special interest and benefit in connection with cottonseed storage, which is more specifically discussed under "Handling and Storage of Cottonseed," 20 pages, by O. H. Alderks. The chapter of 150 pages on "Pigments of Cottonseed" by Charlotte H. Boatner, seems rather lengthy, although it will be of immense value to the scientist or student specializing in this field.

The material under "Cottonseed Oil," 44 pages, by the editor, Alton E. Bailey, is impressive for its completeness and relative conciseness. Thomas D. Fontaine's chapter, 56 pages, on "Cottonseed Proteins" deals with chemical and physical properties; and those proteins constituting the primary commercial value of cottonseed meal and cake are treated from the standpoint of their nutritional significance under "Cottonseed as a Source of Animal Feedstuffs," 42 pages, by Fred Hale and Carl M. Lyman. Under "Miscellaneous Constituents," by F. G. Dollear and K. S. Markley, there are 27 pages on chemical properties and physical characteristics of linters, hulls, and kernels. The portion on sampling under "Grading and Evaluation of Cottonseed," 30 pages, by Guy S. Meloy, should be of special interest to the chemist. The material on sampling, methods of analysis, and collaborative check work is also of unusual interest under "Grading and Evaluation of Cottonseed Oil, Cake, and Meal," 23 pages, by E. R. Barrow. Another chapter of 12 pages by Guy S. Meloy, covers "Grading of Cotton Linters." The details of the technology of cottonseed processing are well presented in the following chapters: "Mechanical Pretreatment of the Seed," 21 pages, by A. Cecil Wamble; "Cooking of Meats and Recovery of the Oil," 30 pages, by O. H. Alderks; and "Economics of Cottonseed Crushing," 37 pages, by John F. Moloney.

A chapter on "Processing of Cottonseed Oil," 40 pages, by Edward M. James, is followed by one on "Edible Cottonseed Oil Products," 29 pages, by Howard C. Black, which covers particularly well the various types of shortening for specific bakery uses. Another chapter of nutritional importance deals with the role of fat in human nutrition under "Nutrition Aspects of Cottonseed Oil Utilization," 48 pages, by Harry J. Deuel, Jr. Information is furnished on the relatively small proportion of cottonseed oil entering the non-edible field, under "Non-edible Cottonseed Oil Products," 13 pages, by O. H. Wurster, W. J. Govan, Jr., and G. J. Stockmann. A few pages are devoted to "Miscellaneous Products from Seed and Meal," by A. E. Bailey, which include some information on cottonseed flour and its use. The two remaining chapters are on "Cottonseed Hulls," 20 pages, by John W. Dunning, and "Cotton Linters," 11 pages, by Peter Van Wyck.

"Cottonseed and Cottonseed Products" encompasses a wide variety of subjects pertaining to the cottonseed industry. It is very readable and well illustrated, and the typography is good. Although some sections will be more useful than others, the reviewer recommends this book to anyone engaged in the cottonseed industry.

V. E. MUNSEY

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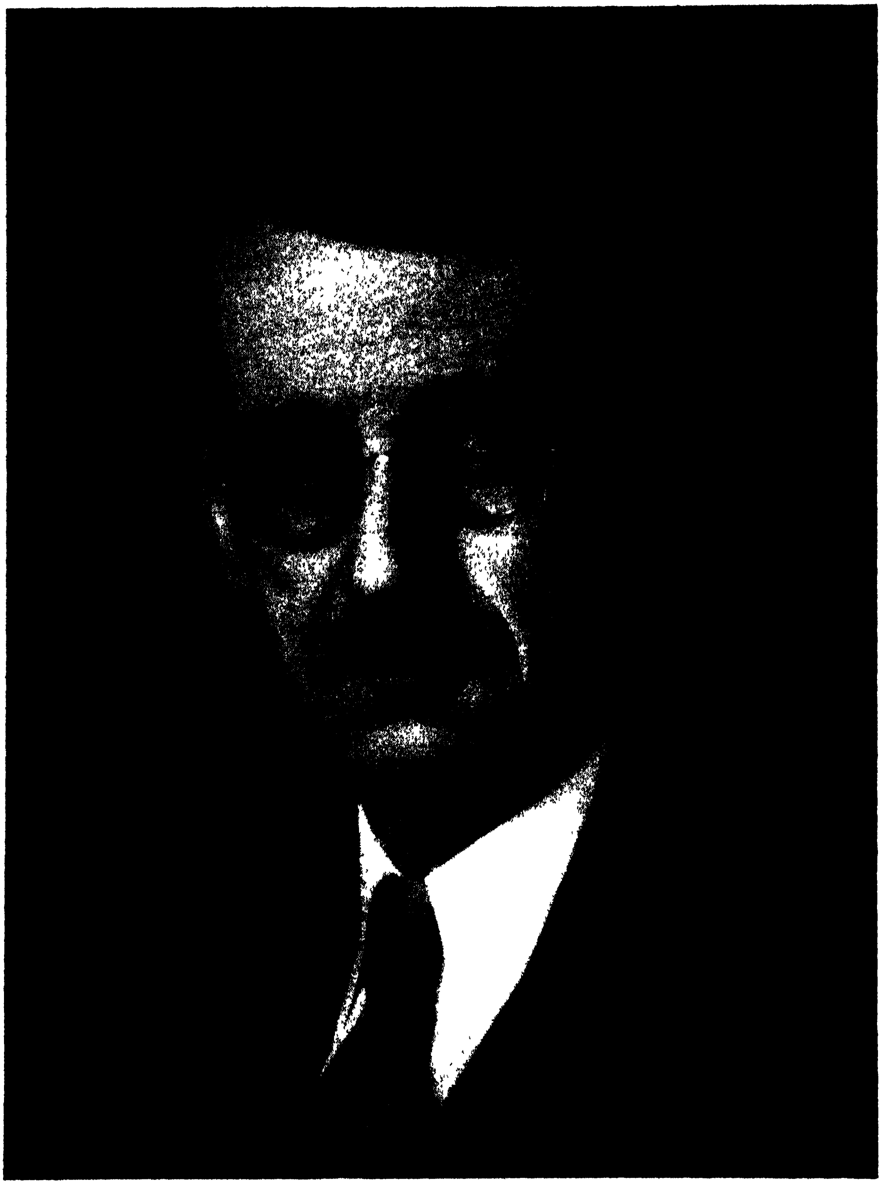

Edward Monroe Bailey, 1879–1948

EDWARD MONROE BAILEY

Edward Monroe Bailey died at his home in New Haven, Connecticut, on April 13, 1948, after an illness of several months. He was born at New London, Connecticut, on August 27, 1879, the son of Edward Monroe and Louise Maria (Hagan) Bailey. His father moved to a farm at Higganum, Connecticut, when he was a few months old, and it was here that Bailey grew up, attending local schools and graduating from the Middletown High School in 1898. It was never characteristic of Monroe Bailey to talk about himself and he revealed none of the details of his earlier life to his associates. Mrs. Bailey is responsible for one incident of interest: a friend offered to pay his expenses at Wesleyan University if he would agree to become a minister, but he declined.

After a short interlude as a country school teacher he entered the Sheffield Scientific School of Yale University and graduated in 1902. Immediately after his graduation (July 1902) he came to work at the Connecticut Agricultural Experiment Station as an assistant chemist and remained continuously at the Station thereafter until his retirement on October 1, 1945, a total of 43 years. In 1918 he was appointed acting head of the Department of Analytical Chemistry in the absence of J. P. Street, then in military service; the following year Mr. Street resigned and Bailey received the permanent appointment as chemist in charge that he held for the ensuing 26 years.

While working at the Station Bailey continued his advanced studies at Yale, receiving the master's degree in 1905 and the doctorate in 1910. His work under the late Lafayette Mendel on the composition of the banana was published in a series of articles in the Journal of the American Chemical Society and the Journal of Biological Chemistry.

He was married to Myrtle Mix Studley of New Haven on June 11, 1906. One son, Irving Monroe, was born on August 13, 1914, and is now living in Hartford. There is also a young granddaughter, Beverley.

Bailey's published papers covered a variety of investigations into the chemistry and analysis of foods and drugs: the studies on the banana previously mentioned, the analysis of noodles, the determination of vanillin, coumarin, and acetanilid in vanilla extract, the detection of sulphites in meat, the determination of starch in meat, tests for the anthraquinone drugs and a study of the carbohydrates and enzymes of the soy bean. He acquired a very extensive knowledge of the composition of common and uncommon foods, and it was under his direction that the Connecticut Agricultural Experiment Station undertook the analyses of nearly all the foods on the market that were frequently reproduced in diabetic manuals before the advent of insulin. He was responsible for the compilations of analyses of foods that appeared in several editions of Joslin's "Treatment of Diabetes Mellitus."

It was probably a combination of this familiarity with the composition of foods with his reputation for good judgment that was responsible for his appointment to two positions that he held for many years. In 1924 he was appointed by the A.O.A.C. as a member of the "Committee to Coöperate with Other Committees on Food Definitions," and from that date he served on the old Food Standards Committee until 1940, when the new U.S. Food Drug, and Cosmetic Act authorized the promulgation of standards that were no longer purely advisory. He probably would have been appointed to the new Standards Committee also had his health permitted. In 1930 he was appointed a member of the Council on Pharmacy and Chemistry of the American Medical Association and continued to serve until 1938. When this Council set up a Committee on Foods in 1931 he was an original member and remained on the committee when it became a separate Council in 1936, resigning in 1938.

Almost from the first day he went to work for the Experiment Station Bailey took an active part in the work of the A.O.A.C. At the first meeting he attended—in 1904—he was appointed Associate Referee on Fruit Products, and the following year he presented a report on the determination of water in dried fruits. At various times he held refereeships on dairy products (1913), tea (1918–1919), cacao products (1924 and 1926) and coffee (1932–1933) and associate refereeships on cocoa and cocoa products (1906), the cryoscopic examination of milk (1921), alcohol in drugs (1928) and colorimetric methods for vitamins (1929). He was a member of Subcommittees B and C, and served as Chairman of the Committee on Recommendations of Referees continuously from 1926 to 1934. He was a member of the Executive Committee in 1923–1924, 1926–1928 and 1931, and was vice president of the Association in 1929 and President in 1930.

The fourth and fifth editions of the "Book of Methods" were prepared under his editorial direction; he was delighted when the Association showed its appreciation of the quality of these two compilations by presenting him with a silver service.

As chairman of the Committee to Confer with the American Public Health Association on Standard Methods of Milk Analysis from 1932 and 1943 he was responsible for the chemical section of several editions of that manual of the A.P.H.A.

His Connecticut duties included responsibility for administration of the feed and fertilizer laws. The laboratory under his direction not only made the usual chemical analyses of feeds but determined the ingredients microscopically and assayed vitamin D supplements by animal feeding. When the Association of American Feed Control Officials was organized he took an active part in this association as well as in the A.O.A.C., and in 1934 he served as its president.

This recital of Bailey's scientific accomplishments and the positions he held leaves out the most important qualities of the man himself. What will most be remembered about him by those who worked under him can be expressed in one simple word—his "goodness." I have never met a person who had one ill word to say about him. He did not tolerate slipshod chemical work or mental dishonesty in his subordinates, but he had a never failing friendliness and helpfulness. When the present writer was in the hospital for a serious operation some years ago there was never a day that Bailey did not drop by to see him. This kindliness and his excellent judgment were his outstanding characteristics. His knowledge of the chemistry and analysis of foods was encyclopedic, and this was united with a critical faculty in interpreting analytic evidence that was almost intuitive. In his field he will have few equals and no superiors; his personal character will always be an inspiration to those of us who knew him.

HARRY J. FISHER

TUESDAY—MORNING SESSION

REPORT ON VITAMINS

By CHESTER D. TOLLE (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.), *Referee*

Vitamin A.—The Associate Referee made no report.

Vitamin B_1.—The recommendation of the Associate Referee, which reads as follows, is approved. That the thiochrome method for thiamine be made official, first action.

Vitamin B_2.—The recommendations of the Associate Referee, which read as follows, are approved:

(1) That further work be conducted on the fluorometric method that was the subject of this year's study.

(2) That further consideration be given to improvements that may be made in the present microbiological method.

Vitamin C.—The Associate Referee made no report.

Vitamin D. (*Poultry*).—The Associate Referee made no report.

Nicotinic Acids.—The Associate Referee made no report.

Pantothenic Acid.—The Associate Referee made no report.

Folic Acid.—The recommendation of the Associate Referee, which reads as follows, is approved: That the findings in this investigation of folic acid indicate the need for further study.

Carotene.—The recommendations of the Associate Referee, which read as follows, are approved:

(1) That the collaborative procedure for determination of carotene be further studied, with different samples of alfalfa meal, as well as other types of food and feed materials.

(2) That the collaborative procedure as outlined in his report be adopted as an alternative method for the determination of carotene in hays and dried plants.

No reports were given for vitamin A, vitamin C, or vitamin D (milk, poultry). For recommendations of the Associate Referee on vitamin B^2, see Referee report.

REPORT ON THIAMINE

By O. L. KLINE (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

In 1943 (1) it was recommended to this Association that the U. S. Pharmacopoeial method for the determination of thiamine by the thiochrome procedure be adopted as a tentative chemical method to be applied to cereal products and vegetable products. This recommendation was based upon the results of an extensive collaborative study that had been sponsored by the United States Pharmacopoeial Vitamin Advisory Board. The thiochrome method adopted at that time has served for the past four years as a tentative A.O.A.C. procedure and now appears in the Sixth Edition of the *Book of Methods*. During this time the results of wide experience with the method have demonstrated that it is a satisfactory one in all essential details. There have been a number of studies designed to test the reliability of various steps of the procedure but in the main the method as now used is that described in the 1943 report.

The American Association of Cereal Chemists, in connection with the program of control of the enrichment of bread and flour, has been vitally interested in chemical methods for the determination of thiamine. From a collaborative study reported in 1944 by Andrews (2) in which samples of flour and bread were examined in a large number of laboratories it was concluded that the oxidation of thiamine to thiochrome in the procedure was a critical step that required further study. Last year Watson, (3) of the Food and Drug laboratory at Winnipeg, and independently, Hall of the Vitamin Division laboratory, learned that the order and speed of addition of the reagents and sample solution used in the oxidation step, as well as the degree of shaking of the reaction mixture, are important factors in obtaining a maximum amount of thiochrome. Since less than 100 per cent of thiamine is converted to thiochrome a procedure carefully standardized at this point is extremely important. Watson points out that thiamine may be converted in weak alkaline solution to a carbinol form from which thiochrome is formed slowly. This was described by Zima and Williams (4) in their studies of crystalline thiamine. A strong alkaline solution depresses the conversion to the carbinol form and therefore favors optimum conversion to an activated form of thiamine which is rapidly oxidized by ferricyanide to the fluorescing thiochrome. Hall described before this Association last year studies that indicate that excessive shaking, following addition of the oxidizing reagents to the sample solution, reduces the amount of thiochrome formed because, he postulates, an excessive amount of oxygen is introduced into the solution. He also pointed out that too little shaking led to less than maximum thiochrome formation because of insufficient mixing, and incomplete oxidation. His experiments have further indicated, that in the presence of the amount of acid recommended, the use of 15 per cent sodium hydroxide is sufficient to give a concentration of alkali necessary for maximum conversion to thiochrome. The thiochrome method as it appears in the Sixth Edition of *Methods of Analysis* takes into account the results of these studies.

The American Association of Cereal Chemists has had for the past several years a program of check sample analyses which has included the use of the thiochrome method. It is interesting to note in the report of Meredith (5) which describes the results obtained in the 1944–1945 study, that there was great improvement in the results obtained over those for the previous year.

In the Food and Drug Administration there are four laboratories in which flour and bread are routinely examined for thiamine content by the thiochrome procedure. In these four laboratories the A.O.A.C. method has been closely followed during the past year. In order that each of the analysts involved interpret properly the written procedure, and understand the more critical steps of the method, W. L. Hall of the Vitamin Division visited each of the laboratories and carried through the method with the analyst. To correlate the results obtained in these four laboratories a check sample analysis program has been in effect for some time. Mr. Alfend of the St. Louis Station has been in charge of preparation of these samples and of reporting the results of analysis. For more than a year samples of flour have been used as check samples with the examination of one each month. The results of analysis of 15 check samples are presented in Table 1.

TABLE 1.—*Thiamine values for flour check-samples*
(Assayed in Food and Drug Administration Laboratories)

SAMPLE	LABORATORY			
	I	II	III	IV
		Milligrams per pound		
1	1.99	1.92	2.00	2.28
2	1.83	1.98	1.81	1.89
3	2.23	2.24	2.16	2.21
4	1.87	1.78	1.81	1.98
5	3.10	2.85	3.04	2.95
6	1.70	1.65	1.68	1.53
7	2.41	2.38	2.34	2.51
8	1.64	1.66	1.61	1.65
9	1.94	1.93	1.96	1.98
10	2.40	2.38	2.42	2.25
11	1.18	1.21	1.23	1.15
12	1.80	1.77	1.77	1.77
13	2.20	2.28	2.26	2.31
14	1.88	1.94	1.94	2.01
15	2.30	2.29	2.13	2 15

Average Standard Deviation between laboratories = .075
Coefficient of Variation = ±3.7%

Many types of products may be assayed by the thiochrome procedure with elimination of the base-exchange step. Flour is an example of such a product and it is common practice to omit the use of the adsorption column in its examination. The results in Table 1 were obtained by the shortened procedure. The results given are in milligrams of thiamine per pound, on an air-dry basis. Thiamine content of the samples examined varies from 1.2 to 3.1 mg. per pound. Variation between laboratories has been calculated, by analysis of variance.

As indicated in the table, the coefficient of variation between laboratories is ±3.7%. This means that in 1 of 20 determinations the checks will exceed ±8% of the mean. The agreement between laboratories is exceedingly good for this type of determination, and illustrates the value of personal contact among analysts.

TABLE 2.—*Thiamine values for collaborative bread samples*
(Assayed in Food and Drug Administration laboratories)

SAMPLE	LABORATORY			
	I	II	III	IV
	Milligrams per pound—air-dry weight			
1	1.89	1.92	2.06	1.90
2	1.65	1.69	1.72	1.58
3	1.70	1.72	1.74	1.60
4	1.63	1.72	1.77	1.64
5	1.63	1.69	1.69	1.54
6	0.41	0.43	0.40	0.39
7	0.43	0.43	0.43	0.39
8	0.28	0.29	0.30	0.30
9	0.34	0.36	0.35	0.31
10	0.33	0.34	0.34	0.30

	ALL SAMPLES	SAMPLES 1 TO 5	SAMPLES 6 TO 10
Av. Stand. Dev. between laboratories—	.050	.068	.018
Coefficients of Variation—	±4.8%	±4%	±5%

Bread is a type of product that requires the base-exchange step in its analysis by the thiochrome method. To test the effect of the adsorption technic on uniformity of results from different laboratories, a series of bread samples was prepared and distributed to the four laboratories. Samples of fresh bread were purchased in Washington, and dried to an air-dry weight in the Vitamin Division laboratory. In order to have a range of thiamine values, samples of both enriched and unenriched bread were obtained. These samples were dried at room temperature overnight, then at 50°C for 8 hours in a forced-draft oven. They were then allowed to come to moisture equilibrium in the air-conditioned laboratory, bottled, and distributed. The analytical procedure included the use of the base-exchange column. The thiamine values reported are milligrams of thiamine per pound of bread on an air-dry basis and are presented in Table 2. Samples 1 to 5, labelled "enriched," ranged from 1.6 to 2.0 mg. per pound dry weight. Samples 6 to 10 were unenriched white breads.

Again for this type of determination, remarkably good agreement was obtained between laboratories. For all samples examined the average standard deviation between laboratories was .050 with a resulting coefficient of variation of ±4.8%. This means that 1 in 20 comparisons will exceed ±10 of the mean. Thiamin content of the sample had little effect upon the error of the method, as illustrated by the similarities of coefficients of variation for samples 1 to 5 and 6 to 10.

The results presented here serve as a basis for the following recommendation:

It is recommended* that the A.O.A.C. tentative thiochrome method for thiamine applied to cereals and vegetables be made official, first action.

Acknowledgments: Miss Lila Knudsen and her staff prepared the statistical evaluations of the data prescribed here. Analyses were conducted at the Philadelphia, St. Louis, and San Francisco Stations, and in the Vitamin Division Laboratory. Analyses reported were carried out by W. L. Hall, E. L. Combs, R. M. Hawkins, S. Alfend, V. H. Blomquist, L. H. McRoberts, N. E. Foster, A. M. Fleiss, F. C. Minsker. Bread samples were prepared by H. W. Loy. The efforts of all these are greatly appreciated.

REFERENCES CITED

(1) KLINE, O. L., *This Journal,* **27,** 534 (1944).
(2) ANDREWS, J. S., *Cereal Chem.,* **21,** 388 (1944).
(3) WATSON, H. A., *Ibid.,* **23,** 166 (1946).
(4) ZIMA, O., and WILLIAMS, R. R., *Ber.,* **73B,** 941 (1940).
(5) MEREDITH, W. O. S., *Cereal Chem.,* **23,** 585 (1946).

No reports were given on nicotinic acid or pantothenic acid.

REPORT ON CAROTENE

By F. W. QUACKENBUSH (Department of Agricultural Chemistry, Purdue University Agricultural Experiment Station, Lafayette, Ind.), *Associate Referee*

To find out what was wanted in a carotene procedure and what equipment was available for measuring carotene in the various laboratories of the country, a questionnaire was sent to some 120 control, research and industrial laboratories over the United States and Canada. Most of them replied and their response brought forth a number of significant points, some of which are shown in Table 1.

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* For report of Subcommittee A and action of the Association, see *This Journal,* 31, 44 (1948).

State control laboratories in considerably less than half of the States are attempting to determine carotene in any feed or food products. Most of the research and industrial laboratories contacted were doing analyses.

The replies indicated that present and former A.O.A.C. methods for carotene are not widely used. About one-third of the replies indicated some use was being made of A.O.A.C. procedures and some of these considered them unsatisfactory. Nearly two-thirds favored discarding present A.O.A.C. methods in favor of some other method.

Among the many suggested substitutes, the methods of Wall and Kelley (1) and Silker, Schrenk, and King (2) were most frequently mentioned. Replies agreed almost unanimously that chromatography is an essential step in a satisfactory method. A few indicated they were making use of dichromate standards.

TABLE 1.—*Summary of results from questionnaire*

	STATE CONTROL LABORATORIES		STATE & FEDERAL RESEARCH	INDUSTRIAL	TOTAL
	FEED CONTROL	FOOD & DRUG			
Replies	35	13	21	19	88
Analyzing for carotene	17	1	20	13	51
Wish to collaborate	19	3	17	12	51
Have spectrophotometer	17	3	18	12	50
Have photoel. colorimeter	4	1	2	3	10
Have visual colorimeter	2	3	—	—	5

Spectrophotometers were available in the vast majority of the laboratories which expressed an interest in doing carotene analyses. Most of the remainder had photoelectric colorimeters.

Fifty-one replies indicated a willingness to collaborate on analyses. Some of these suggested names of others who would probably wish to participate. The test materials most frequently suggested were alfalfa—45, carrots—23, commercial feeds—8, and tomatoes—5.

COLLABORATIVE ANALYSES OF ALFALFA MEAL SAMPLES

Guided by the suggestions from the questionnaire, an attempt was made to provide a simple and rapid procedure based on what seemed to be the best features of the methods of Wall and Kelley (1), Silker, Schrenk, and King (2), and Zscheile and Whitmore (3). After several tests by different analysts in our laboratories, a procedure was sent out to 65 laboratories followed by two samples of dehydrated alfalfa meal—one fresh, the other nearly a year old. Our analysts had obtained average carotene values of

296 p.p.m. and 40 p.p.m., respectively, on the two samples. A sample of magnesium oxide adsorbent was included. Instructions were to hold samples at room temperature and to send in results within a week.

COLLABORATORS

State control laboratories: Maine, New Hampshire, Connecticut, Massachusetts, Maryland, Ohio, Kentucky, Indiana, Minnesota, North Dakota, South Dakota, Texas, California, Oregon, and Montreal, Canada.

State and federal research laboratories: Agricultural Experiment Stations of Delaware, Michigan, Kansas, Oklahoma, Utah, Arizona, California; Department of Botany, University of Chicago; Eastern, Southern, and Western Regional Laboratories; U. S. Food and Drug Laboratories at Washington, D. C., and St. Louis; Bureau of Dairy Industry, Beltsville; Fish and Wild Life Service, Bowie, Maryland; Q. M. Food and Container Institute, Chicago.

Industrial laboratories: Best Foods, Inc., Bayonne, N. J.; Food Research Laboratories, N. Y.; G. L. F. Mills, Inc., Buffalo; Eastern States Farmers' Exchange, Buffalo; General Biochemicals, Inc., Chagrin Falls, Ohio; Central Mills, Dunbridge, Ohio; General Mills (Larrowe Division), Rossford, Ohio; Cargill, Inc., Minneapolis, Minn.; Cerophyll Laboratories, Kansas City, Mo.; W. J. Small Co., Kansas City; Ralston-Purina Co., St. Louis; Merchants Exchange Laboratory, St. Louis; Elk Valley Alfalfa Mills, Independence, Kansas; National Alfalfa Dehydrating and Milling Co., LaMar, Colo.; Albers Milling Co., Seattle, Wash.; Laucks Laboratories, Inc., Seattle.

SUMMARY OF COMMENTS OF COLLABORATORS

Many collaborators called attention to two typographical errors (in the instructions), *i.e.* (1) the figure 100 which should have been 1000, in the formula for calculating results and (2) the number of the magnesium oxide adsorbent, given as 2462, which should have been 2642. Several wished the alfalfa samples had been larger. Some reported that a 100 ml. volumetric flask was not large enough to contain the eluate. It was anticipated that all collaborators would attach the chromatographic column to a suction flask and use a good water pump under which conditions in our laboratory, the final volume of eluate was found to be less than 100 ml. in a number of trials. The directions should have been more specific.

COMMENTS OF COLLABORATORS

Some of the more pertinent comments from individual collaborators are listed below:

No. 3.—First 50 ml of eluate, which contained no color, was not collected in the 100 ml flask used for photometry.

No. 6.—The chromatographic separation using the adsorbent provided gave excellent separation of the various bands.

No. 11.—The solutions seemed to pass through the column very slowly. Otherwise the method appears to be easy and quick.

No. 14.—The procedure used met with favorable comment from others in the laboratory. It is not time-consuming and is simple to carry out.

No. 15.—I would prefer to make a greater dilution (200 ml) on samples of high carotene content like Sample No. 1. The 50 ml of wash solution did not remove quite all of the carotene.

No. 17.—We were able to check our results better with the present A.O.A.C. procedure. Otherwise the collaborative procedure is satisfactory. There was very little difference in time required to make the determinations.

No. 18.—Collection of 100 ml of extract and 50 ml of acetone-hexane wash in 100 ml volumetric flask is confusing. With the use of a 1 mm capillary on adsorption column the use of suction would facilitate the passing of the extract through the column.

No. 22.—Petroleum ether (Skellysolve F) used. Carotenes did not pass through column rapidly. The first 25 ml or more of filtrate was colorless and more than 50 ml of wash solvent was needed before the carotene was washed completely through the column. This necessitated a final volume of eluate that exceeded 100 ml.

No. 26.—No difficulties were encountered in using the procedure as given. It would be of interest to know how well it would work with plant tissues other than alfalfa.

No. 27.—2 gm. sample for Sample #1 gave too concentrated an extract. 50 ml washing did not produce a colorless filtrate at end of washing. 100 ml is too much to put through column. Believe an aliquot would be just as effective.

No. 30.—The exact amount of the aliquot used for chromatography should be specified. If the whole extract is used, part of the carotene will come through the column.

No. 31.—The analytical procedure is essentially the same as the one I have been using for some time. It might be helpful if the reasons for adopting 436 millimicrons as the reference point, and for the use of the specific absorption coefficient of 196 were given in detail. The absorption curve shows no inflection at 436.

No. 32.—Preliminary study of the method using our magnesium oxide (Micron Brand #2642) seemed to show that 50 ml. was insufficient eluant to remove the carotene. We therefore ran some tests using larger volumes of eluant. Our adsorbent has different adsorptive power than that furnished with the alfalfa samples.

No. 35.—We find two points in the procedure ambiguous: (a) Under "Chromatography" what is meant by "15 cm of the dry adsorbent?" The amount used is fixed by the description of the column size in the first line, so this statement seems at best only unnecessarily confusing. (b) Also under "Chromatography" we assume that only an aliquot of the extract was to be chromatographed and we have used 50 ml aliquots throughout. This point could be clarified in the procedure.

No. 36.—Our adsorbent develops more quickly with the eluting mixture.

No. 37.—The collaborative procedure gives results in excellent agreement with the procedure used in our laboratory. The extraction procedure in the collaborative method is somewhat inconvenient since it requires constant attention due to bumping and spattering of the meal on the walls of the extraction flask. With certain samples such as silage which give many carotenoid bands, the failure of the collaborative procedure to give a sharp carotene band on adsorption might be a disadvantage. On the other hand, the fact that extracts need not be concentrated is a definite advantage. On the whole, the technique is quite similar to that used in our laboratory for some time and we think that it has many advantages over the other methods previously used in A.O.A.C. studies.

No. 37a.—The logic of using alpha $=196$ at 436 mμ should be given careful consideration. Personally I do not like chromatography where the band of interest does not segregate. Although the results may seem to be satisfactory, the excesssive eluant (acetone) present may also elute extraneous material in an unknown fashion. The method we prefer makes of alcoholic KOH saponification petroleum extraction and elution on a 3 MgO to 1 of Celite or sometimes a 1:1 mixture. Segregation and separation are then under visible control. Our procedure uses small columns and takes about the same total time as the one you have submitted.

No. 40.—30 ml acetone-hexane for original extraction can go dry easily if condenser doesn't fit snug. Directions have inverted numbers designating MgO brand number 2642. The 10 cm adsorption column is fully twice as long as necessary. This may be good precaution in a test set of this type. Final volume of eluate is 100 ml. This appears larger than necessary and makes the density value unnecessarily low.

No. 41.—With the sample high in carotene, we encountered some difficulty in the elution of pigments other than carotene through the column.

No. 42.—The procedure appears to work quite well. A shorter column seems to speed up the operation. The procedure given or some variation of it appears to be a great improvement on past collaborative procedures. Would suggest a larger sample for future work.

No. 46.—Method very satisfactory.

No. 50.— . . . the outlined method of chromatography is too time-consuming to be practical.

No. 53.—The eluate from #1 sample was made to a volume of 250 ml in order to keep the density readings in a normal position on the Beckman transmission scale.

No. 55.—Final solution from sample 1 was made up to 250 ml volume as original solution was too concentrated for accurate reading. 50 ml wash solution appears as perhaps inadequate for some samples. However, a greater volume of wash solutions with the acetone of specified concentration would probably cause inaccuracy due to xanthophylls which might be eluted.

No. 56.—Collaborative procedure more rapid than present method.

No. 57.—Too little solvent for refluxing gave erratic results. Not necessary to pass all of sample through adsorption column.

No. 60.—Your method of assay for dried vegetable material is rapid and easy to carry out.

RESULTS AND DISCUSSIONS

Analyses were made in 49 laboratories, 41 of which used the collaborative procedure. The individual results are shown in Table 2. The average results are grouped by type of laboratory and type of instrument and compared with results from other procedures in Table 3.

The individual results showed fairly good agreement. It is suspected that some of the low values resulted from placing the sample in a thimble during extraction. The directions were apparently not emphatic enough on this point. Some of the high results can be attributed to erroneous standards.

Results from the different types of laboratories showed similar deviations but remarkably good agreement in average values. No significant trend of values was shown for any of the different instruments used.

The results compared favorably with those obtained by two other methods but were generally lower than those obtained by past or present tentative A.O.A.C. methods.

TABLE 2.—*Individual results of collaborative study on alfalfa meal samples*

A. State control laboratories:

COLL. NO.	INSTRUMENT	COLL. METHOD		OTHER METHODS OR MODIFICATIONS		
		SPLE. I	SPLE. II	SPLE. I	SPLE. II	METHOD
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	
1	Visual colorimeter			325	58	*A.O.A.C.*
3	Beckman	254	38			
4	Visual colorimeter			254	58	*A.O.A.C.*, p. 600
5	Coleman 11	320	53	318	—	*Analyst* **66**, 334 (1941)
6	Beckman	277	28	294	13	*Science*, **90**, 623 (1939)
7	Nessler tubes			292	49	Thimble used
9	Coleman 11			276	36	Wall-Kelley[1]
11	Cenco-Sheard	271	39			
12	Beckman	284	42			
14	Beckman	311	38			
15	Klett-Summerson	274	35	289	25	Wall-Kelley[1]
17	Beckman	272	38	264	45	*A.O.A.C.*
18	Beckman	248	38			
22	Evelyn	314	41	304	59	*A.O.A.C.*, 1940 Ed.
24a	Beckman	296	40			
	Av.	284	39			
B. State and federal research laboratories:						
25	Beckman	282	37			
26	Beckman	307	45			
	Cenco-Sheard			275	43	*A.O.A.C.*, p. 145
27	Beckman	295	46	297	—	"Methods of Vitamin Assay"
29	Beckman	312	33			
30	Evelyn	308	44	286	21	Cold extraction overnight
31	Beckman	317	24			
32	Beckman	287	36	299	—	Column washed 100 ml. of solvent
32	Evelyn	296	36	—	45	Sample rehydrated
32				281	49	*Ind. Eng. Ch.* **35**, 1173 (1943)
33	Beckman	288	39	278	35	Silker, *et al.*[2]
34	Evelyn	299	34			
35	Beckman	264	38			
36	Klett-Summerson			282	32	Thimble used
37	Beckman	304	45	304	48	Wall-Kelley[1]
37a	Beckman	309	47	230	32	Different calculation
38	Hilger			282	30	Thimble used
38	Hilger			300	58	*Ind. Eng. Chem.* **13**, 600 (1941)
39	Evelyn	330	36			
40	Klett-Summerson	272	35			
	Av.	298	38			

C. *Industrial laboratories:*

COLL. NO.	INSTRUMENT	COLL. METHOD		OTHER METHODS OR MODIFICATIONS		
		SPLE. I	SPLE. II	SPLE. I	SPLE. II	METHOD
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	
41	Cenco photel.	301	51	270	40	Silker, *et al.*[2]
42	Beckman	298	29			
43	Coleman 11	256	35			
44	Klett-Summerson			352	48	*A.O.A.C.*, p. 600
46	Lumetron 402 EF	263	30			
47	Beckman	273	33			
48	Beckman	270	30			
49	Klett-Summerson	288	42			
50	Klett-Summerson	309	44	307	29	Soda ash chromatog. after 72 hr. extn. with p.e.
51	Evelyn	324	56	360	68	*A.O.A.C.*, 1945
52	Beckman	286	25	282	25	Extn. 1 hr. on mech. shaker
53	Beckman	353	49	286	35	Wall-Kelley[1]
55	Klett-Summerson	290	35	291	35	Silker, *et al.*[2]
56	Coleman 11	287	39	270	53	Wall-Kelley[1]
57	Evelyn	266	33	283	36	25 ml. chromotographed
60	Beckman	307	38	340	48	By chart from carotene std.
	Av.	292	38			

TABLE 3.—*Comparison of results by groups*

A. *Collaborative procedure:*

	NUMBER OF CASES	AV. VALUES ON SPLE. I	SPLE. 2
1. Type of laboratory—			
State control	11	285	39
State & Federal research	15	298	38
Industrial	15	292	38
	41	Av. 292	38
2. Type of instrument—			
Beckman	23	291	37
Evelyn	7	305	40
Klett-Summerson	5	287	38
Coleman 11	3	287	42
Other	3	278	40

B. *Other procedures:*

A.O.A.C.	6	310	56
Wall-Kelley	5	285	39
Silker, *et al.*	3	280	37

RECOMMENDATIONS*

It is recommended—

(1) That the collaborative procedure be studied further with different samples of alfalfa meal as well as other types of food and feed materials.

(2) That the collaborative procedure as outlined for the determination of carotene in hays and dried plants be adopted as an alternative tentative method. Complete details of the methods are given in *This Journal*, **31**, 111–112 (1948), under "Changes in Methods of Analysis."

REFERENCES

(1) Wall, M. E., and Kelley, E. G., *Ind. Eng. Chem.* (*Anal. Ed.*), **15**, 18 (1943).
(2) Silker, R. E., Schrenk, W. G., and King, H. H., *Ibid.*, **16**, 513 (1944).
(3) Zscheile, F. P., and Whitmore, R. A., *Ibid.*, **19**, 170 (1947).

REPORT ON FOLIC ACID

By Laura M. Flynn (Dept. of Agricultural Chemistry, University of Missouri, Columbia, Mo.), *Associate Referee*

Folic acid in natural products has been assayed both biologically and microbiologically. The chick is commonly used for biological assays and usable procedures have been described by O'Dell and Hogan (1), Campbell, Brown, and Emmett (2), Hutchings, Oleson, and Stokstad (3), Scott and coworkers (4), and Lillie and Briggs (5). Day and Totter (6) have recently published an excellent review of published methods for the bioassay of the "Vitamin M group" (the factor variously called vitamin M, factor U, vitamin B_c, norite eluate factor, folic acid, and *L. casei* factor).

Not long ago, Stokstad and Hutchings (7) prepared an excellent summary of published procedures for the microbiological assay for this vitamin, and more recently Roberts and Snell (8) described a modified medium that should improve materially the assay procedure for pteroylglutamic acid. When *L. casei* is used as the test organism, the excess of strepogenin furnished in this new medium in the form of trypsinized casein should insure rapid early growth of the organism, thus overcoming one of the main objections to the use of *L. casei*. The identification of this new vitamin and the elucidation of the chemical structure of pteroylglutamic acid were greatly speeded by microbiological assays with lactic acid bacteria as test organisms. If, however, one reads the rapidly expanding literature in this field, it is apparent that "no rigorously tested or widely accepted method for quantitative extraction or determination of folic acid is yet available" (8). Kidder and Dewey (9) suggest that the protozoan *Tetrahymena* may be used in testing for folic acid, and they mentioned that Dr. Bird, of Parke, Davis, and Company, is working on an assay method with this organism.

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* For report of Committee A and action by the Association, see *This Journal*, **31**, 44 (1948).

The collaborative study of the assay of folic acid made this year (1947) is the first undertaken under the auspices of the Association of Official Agricultural Chemists. For this study the microbiological procedure of Teply and Elvehjem (10), using *L. casei* as the test organism, was selected from the methods available in the literature. The constituents of this medium are found in most laboratories and the method has given good results in many hands. Detailed directions for the method were sent to all laboratories which had expressed willingness to participate. Chemists co-operating in the study were asked to assay the samples by any other methods which they were using routinely. The materials chosen for assay were (1) dehydrated, powdered spinach, and (2) ground dehydrated and defatted beef (from the round). Each laboratory co-operating in the project supplied its own folic acid standard, bacterial culture, and enzyme preparation, as well as all ingredients for the basal media. We realized that this policy increased the probability of variations in results even though specified and detailed directions were followed explicitly. Two laboratories, at the University of Missouri[1] and the University of Maryland,[2] agreed to assay the spinach by the chick method.

COLLABORATORS

The chemists co-operating in this study are listed below. Grateful acknowledgement is made of the generous and gracious help of all who took part in the endeavor.

O. D. Bird, Parke, Davis, and Company, Detroit, Mich.

H. W. Cromwell, Abbott Laboratories, North Chicago, Ill.

M. C. Caswell and E. Konopka, Merck & Company, Inc., Rahway, N. J.

Laura M. Flynn, Department of Agricultural Chemistry, University of Missouri, Columbia, Mo.

C. M. Hayes, Ayerst, McKenna, and Harrison, Ltd., Station "O," St. Laurent, Montreal, Province of Quebec, Canada.

Lloyd Hein, Wisconsin Alumni Research Foundation, Madison 6, Wis.

T. H. Jukes, Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.

Dorothy J. Longacre, National Dairy Research Laboratories, 1403 Eutah Place, Baltimore 17, Md.

Henry W. Loy, Jr., Vitamin Division, U. S. Food and Drug Administration, Washington, D. C.

Bernard L. Oser, Food Research Laboratories, Inc. 48-14 Thirty-third Street, Long Island 1, N. Y.

L. R. Richardson, Department of Biochemistry and Nutrition, Agricultural & Mechanical College of Texas, College Station, Tex.

H. S. Schaefer, Ralston Purina Laboratories, St. Louis 2, Mo.

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[1] Boyd L. O'Dell, Department of Agricultural Chemistry, University of Missouri, Columbia, Mo.

[2] George M. Briggs, Department of Poultry Husbandry, University of Maryland, College Park Maryland.

Ruth Steinkamp, John R. Totter and Paul L. Day, Department of Biochemistry, School of Medicine, University of Arkansas, Little Rock, Ark.

F. M. Strong, Department of Biochemistry, University of Wisconsin, Madison, Wis.

Virginia R. Williams, Louisiana State University, Baton Rouge, La.

L. D. Wright, Sharp and Dohme, Inc., Glenolden, Pa.

RESULTS

The data submitted by the co-operating laboratories are summarized in Tables 1 and 2. To facilitate comparisons, numerical averages and median values are listed in Tables 1 and 2. In the calculation of these averages

TABLE 1.—*Results of 1947 A.O.A.C. collaborative study on folic acid*
(Assays by Teply and Elvehjem Method using *Lactobacillus casei*)

LAB. NO.	SAMPLE I		SAMPLE II	
	SPINACH*		BEEF	
	FREE	TOTAL (CORRECTED FOR ENZYME)	FREE	TOTAL (CORRECTED FOR ENZYME)
	mmg/g	*mmg/g*	*mmg/g*	*mmg/g*
1	7.48	14.9	0.24	0.42
2	4.5	11.5	0.21	0.18
3	4.88	12.9	0.221	0.269
4	*Std. #1*		*Std. #1*	
	4.42	9.47	0.19	0.20
	4.26	9.64	0.22	0.21
	Std. #2			
	7.02	15.03	0.30	0.32
	6.76	15.30	0.35	0.33
5	5.25	12.0	0.25	1.01
6	12.4	29.8	0.57	0.59
7	10.5	*A.O.A.C.* Enz. Treatment 7.8; Routine Enz. Treatment 10.4	.13	*A.O.A.C.* Enz. Treatment 0.20; Routine Enz. Treatment 0.27
8	4.6	12.6	0.28	0.23
9		11.64		
	7.90	13.9	0.140	0.247
10			0.3	0.5
11	5.91	13.8	0.3	1.41
14	6.00	14.4	0.74	1.00
15	7.66	13.7	0.264	0.32
16	9.5	17.0	0.36	0.33
Mean	6.59	12.89	0.282	0.47
Median	(6.00–6.89)	(12.9–13.7)	0.264	0.325

* Result of assay by chick method:
Laboratory A—11 mmg/g
Laboratory B—16 mmg/g

and medians only one figure was used from each laboratory, an average of the several figures from each laboratory as shown in the tables.

Data from Laboratory 6 were not included in computing averages. The Associate Referee inadvertently sent to this laboratory, for use as a standard, a preparation of folic acid which had been partially inactivated by over-heating. Laboratory 4 assayed each sample twice for free folic acid, and twice for total folic acid with each of two folic acid standards. Standard No. 1 and Standard No. 2 were from two different preparations of crystalline folic acid, and it was assumed that Standard No. 1 was better. The results from these two laboratories focus our attention upon the importance of securing a reference standard for folic acid at the earliest possible date.

TABLE 2.—*Results of 1947 A.O.A.C. collaborative study on folic acid*
(Assays by methods other than that of Teply and Elvehjem)

LAB. NO.	SPINACH*		BEEF	
	FREE	TOTAL	FREE	TOTAL
	mmg/g	*mmg/g*	*mmg/g*	*mmg/g*
2	4.6	10.8	0.19	0.17
3	—	11.5	—	0.275
5	3.3	6.7	0.13	0.78
7	12.1	9.4	.13	.28
		8.4		.18
8	4.2	12.3	0.30	0.25
	5.2	11.4	0.33	0.30
9	—	16.8	—	0.383
10	3.6	3.2	0.4	0.5
	3.9	5.6	0.5	0.6
11	10.38	14.48	0.24	0.97
12	4.96	14.61	0.31	0.41
15	7.55	12.99	0.233	0.333
	—	12.35	—	0.26
16	10.35	17.25	0.32	0.32
17	3.48	12.48	0.243	0.37
Mean	6.52	11.86	0.26	0.42
Median	4.6	11.5	0.243	0.32
	4.96	12.3	0.30	0.333

* Result of assay by chick method.
Laboratory A—11 mmg/g
Laboratory B—At least 16 mmg/g

Each value listed for Laboratory 7 is an average of twelve tests. This laboratory subjected one series of twelve samples to the enzyme treatment specified in the directions sent to collaborators, and subjected a second series of twelve samples to the enzyme treatment routinely used in the laboratory. Weighings were made upon three different days. All extracts from both enzyme treatments were assayed according to the Teply and Elvehjem procedure as prescribed in the directions and also by the assay procedure routinely used.

A comparison of Tables 1 and 2 shows that as a rule when a laboratory carried out an assay by more than one method, all methods gave approximately the same results. Occasionally this was not true. Several laboratories reported values showing marked variations resulting from several methods as used in each laboratory.

One of the laboratories making a chick assay on the spinach reported 11 mmg. per gram; the other reported 16 mmg. per gram.

INFORMATION FROM QUESTIONNAIRES

Much helpful information was obtained from the questionnaires returned by the collaborators with their reports. A brief summary of this information follows.

Sensitivity of L. casein cultures.—Eight collaborators stated that they have encountered strains of bacteria identified as *L. casei* which are insensitive to folic acid or have lost their sensitivity with subculturing.

Routines for stock cultures.—Since it seemed possible that subculturing may cause changes in the requirements of *L. casei* for folic acid or unidentified growth factors, or both, an inquiry was made concerning precautions routinely taken to prevent changes in the culture of this test organism. Four of the laboratories commented that they use no special precautions. Six laboratories gave more or less detailed accounts of routines used in the maintenance of stock cultures of *L. casei.* These routines varied appreciably from laboratory to laboratory and their differences may well be reflected in varying responses of the stock cultures to folic acid or to the unidentified growth stimulants found in feeds and foodstuffs. Descriptions were given for four modifications of the agar medium for storage. The frequency of making transplants to agar varied from once a week to once a month. The laboratories choose differing formulas for preparation of liquid media for subcultures from agar.

Size of inoculum.—Methods routinely used for standardizing the inoculum in the several laboratories also show wide variations. About half of the laboratories reporting use 24-hour cultures; others prefer 15- to 18-hour cultures. Arbitrarily assuming that the inoculum cultures contained 10^8 bacteria per milliliter the Associate Referee calculated that various reporting chemists used for seeding a 10-milliliter culture inocula of the following different bacterial concentrations: 4×10^4, 2×10^5, 4×10^5, 1×10^6, 8×10^6, 1.5×10^7, and 3×10^7 bacteria.

L. casei vs. S. faecalis.—Most of the group routinely using *L. casei* prefer to use titration measurements in determining growth response. The group routinely using *S. faecalis* prefer turbidity measurements in determining the response to the vitamin. Half of the chemists reporting prefer *L. casei* as a test organism, the other half prefer *S. faecalis.*

Most laboratories carry stock cultures of *L. casei* for use in riboflavin tests. It appears that *L. casei* is more sensitive to folic acid than is *S. faecalis. L. casei* grows in proportion to the folic acid content of the medium over a range of from approximately 0.75 times 10^{-5} mmg to at least 10 times 10^{-5} mmg of the vitamin per milliliter of culture. Turbidity reading can frequently be made over a much longer range. With media in which a low blank can be secured one can obtain excellent acid production with this organism and the dose-response curve is steep. *L. casei,* being more sensitive to the vitamin, can be used for assays of extracts which are very low in folic acid. The size of inoculum is more critical with *L. casei* than with *S. faecalis.*

With *S. faecalis* the amount of vitamin needed for a quantitative response is about ten times as much as with *L. casei*. Because of its lower sensitivity to the vitamin *S. faecalis* gives low blanks. There may be some difficulty with caramelization of the glucose in a citrate buffered medium for *S. faecalis*, unless the sterilization time is cut considerably. *S. faecalis* does not respond to folic acid conjugates.

Response in "Standard" cultures.—Collaborators were asked whether they found, with the Teply and Elvehjem medium, that the cultures from unknowns grow out more rapidly than cultures containing only standard folic acid solutions. They were also asked what corrections or adjustments they make if the unknowns show more rapid growth. Two of the group replying have had no difficulty with slowly growing standards; seven have made no observations on this point. Eight chemists indicated that under the conditions in their laboratories the cultures containing standard folic acid show a lower rate of early growth than is shown by cultures containing test extracts, with their almost inevitable unknown growth stimulants. Suggested remedies included assaying in reduced concentration and increasing the length of the incubation period. Several laboratories reported excellent results when enzyme digested casein is used. They found it possible with this material to obtain low blanks, more linear standard curves, and maximum growth at high levels of the vitamin.

Another inquiry on the questionnaire was concerned with the amount of acid produced during 72 hours' incubation as compared with the amount produced during an incubation period of 96 to 108 hours. Five replies indicated that the data on this point were insufficient to justify an opinion. One laboratory found no increase in acid production on lengthened incubation. Four replies indicated that acid production at the end of 96 to 108 hours' incubation was appreciably greater than at the end of 72 hours. Two of these four found that the increases in the standards and in the unknowns were so similar that either incubation period could be used with confidence in an assay. On the other hand, two laboratories reported that acid production in cultures containing standard solutions showed more change with increased incubation than was shown by cultures containing extracts of unknowns.

It was the consensus of opinion that norite-treated peptone is not satisfactory as a source of unidentified growth factors for *L. casei* in folic acid assays. The blanks were high, and the dose-response curve was not steep.

Dose-response curves.—The laboratories participating in this study were asked to submit specific data showing dose-response in cultures containing known amounts of pteroylglutamic acid. Data from the different laboratories showed appreciable variation in dose-response curves. With an ideal method and perfect techniques this would not be the case. Reports from several laboratories indicated that they were using the procedure of logarithmic plotting of dose-response curves as suggested by Wood (13) for evaluating their data and making calculations. It is the experience of the Associate Referee that the use of data in logarithmic form, either as numerical logarithms or by the use of graph paper with a log-log grid, is of great aid in the criticism and in the interpretation of microbiological assays.

DISCUSSION OF RESULTS

Each laboratory that participated in this study (1947) procured its own supplies of the ingredients necessary for these assays, including folic acid for a standard, the test organism, peptone as a source of the unknown growth stimulants, and the enzyme preparation for freeing bound folic acid. Under the circumstances the circumstances the assay results as reported in Tables 1 and 2 show, as a rule, fairly good agreement.

Furthermore, we find agreement among the laboratories upon the following points:

(a) *Turbidity tests.*—Those who use *S. faecalis* for folic acid assays find that the amount of the vitamin can be estimated satisfactorily within 16 or 18 hours from the turbidity readings. Those who use *L. casei* find that the turbidity measurement lacks precision when the organism is grown in the Teply and Elvehjem medium. Titration of *L. casei* cultures at the end of 72 hours gives acceptable results.

(b) *Growth in "Blank" cultures.*—When the directions were followed there was too much growth in cultures to which no folic acid was added. In other words, the blanks were too high.

(c) *Need for improvement in method.*—When *L. casei* is the test organism improvements in the medium are desirable. Results were not reliable and reproducible when the specified procedures were followed.

We find disagreement among the laboratories upon the following points:

(a) *Purification of folic acid for standard.*—Several suggestions were made which indicated that the synthetic pteroylglutamic acid used as a standard had been subjected to recrystallization, or other purification procedures before use in a standard solution. Further study is needed to determine what procedures, if any, should be followed to prepare commercial pteroylglutamic acid for a reference standard. Steps looking toward the development of a reliable reference standard have been taken by the proper authorities. In the meantime it would seem wise, whenever possible, to characterize solutions to be used as standards by data on their absorption spectra at several wave lengths. Physical constants for folic acid, including extinction data, have been published by Parke, Davis, and Company (11) and by the Lederle Laboratories (12).

(b) *Choice of test organism.*—Seven of the collaborators expressed a marked preference for *S. faecalis.* Seven prefer *L. casei.* Three commented either that they have not tried *S. faecalis* or that their experience was too limited to justify an opinion. Only a few laboratories indicated that they have used both. Possibly the preferences expressed are the results in some cases of greater experience in the use of one or the other organism in microbiological assays.

Turbidity versus titration measurements.—One would expect preference for the method of evaluating growth response to vary from laboratory to laboratory. Such preference would depend upon the facilities of the laboratory, the extent of the technician's experience with each method, and the standardization and skill attained in the use of the method in any individual laboratory.

Routines for maintenance of stock culture, and preparation of inoculum.—The seemingly minor variations in these routines, as followed in the several laboratories, may or may not be of significance in their effect upon the response of the test organism to the vitamin, or upon the rapidity of early growth in cultures containing standard folic acid.

Basal media for test.—Several laboratories have developed basal media of their own or made adaptations of published media. More extensive comparative tests are needed in order to select or develop the best possible basal medium for tests for this vitamin.

SUMMARY

The microbiological method for determining folic acid was subjected to collaborative assay in 1947, and results showed fair agreement between the laboratories. However, the findings in this first collaborative investigation of folic acid indicate the need for further study of its determination.

REFERENCES

(1) O'Dell, B. L., and Hogan, A. G., *J. Biol. Chem.*, **149**, 323 (1943).

(2) Campbell, C. J., Brown, R. A., and Emmett, A. D., *Ibid.*, **152**, 483 (1944).

(3) Hutchings, B. L., Oleson, J. J., and Stokstad, E. L. R., *Ibid.*, **163**, 447 (1946).

(4) Scott, M. L., Norris, L. C., and Charkey, L. W., Daniel, L. J., and Heuser, G. F., *Ibid.*, **164**, 402 (1946).

(5) Lillie, R. J., and Briggs, G. M., *Poultry Science*, **26**, 289 (1947).

(6) Day, P. L., and Totter, J. R., "The Bioassay of the Vitamin M Group," in Dann and Satterfield, "Estimation of the Vitamins," *Biological Symposia*, *XIII*, 313 (1947).

(7) Stokstad, E. L. R., and Hutchings, B. L., "The Microbiological Assay of *Lactobacillus casei* Factor (Vitamin B_c, Folic Acid)," in Dann and Satterfield, "Estimation of the Vitamins," *Biological Symposia*, *XII*, 339 (1947).

(8) Roberts, E. C., and Snell, E. E., *J. Biol. Chem.*, **163**, 499 (1946).

(9) Kidder, G. W., and Dewey, V. C., *Proc. Natl. Acad. Sciences*, **33**, 95 (1947).

(10) Teply, L. J., and Elvehjem, C. A., *J. Biol. Chem.*, **157**, 303 (1945).

(11) Bloom, E. S., Vanderbilt, J. M., Binkley, S. B., O'Dell, B. L., and Pfiffner, J. J., *Science*, **100**, 295 (1944).

(12) Stokstad, E. L. R., Hutchings, B. L., and Subbarrow, U., *Annals of the New York Acad. of Science*, **48**, 261 (1946).

(13) Wood, Eric C., *Analyst*, **72**, 84 (1947).

PRESERVATIVES AND ARTIFICIAL SWEETENERS

By Margarethe Oakley (State Department of Health, Baltimore 18, Md.), *Referee*

This chapter is alive and now progressing in full vigor. During the past year nine subjects were assigned for study and from the progress made it is estimated that in another two years the chapter will be as up to date as the other subjects in the *Methods of Analysis*.

There is one more topic that should be added for study—namely, the new sweetener, 1-propoxy 2-amino 4-nitrobenzene. Investigations are already being conducted on its toxicity and recently a method for its determination was published (*Chem. Weekblad*, **43**, 283 (1947), through *C. A.* **41**, 5058 (1957).

Benzoate of Soda and Esters of Benzoic Acid

The Associate Referee reported plans to work on a method for the determination of ethyl vanillate; a sample of which he has recently secured.

Saccharin

The Associate Referee conducted tests on several methods for the extraction of baked goods but none proved sufficiently successful to submit to collaborative study.

Quaternary Ammonium Compounds

The Associate Referee completed collaborative studies on quaternary ammonium compounds in commercial preservatives, table sirup, bottled beverages containing fruit juice, and beer. He plans further studies on fruit juices, bottled sodas, milk, salad dressings, and pickled relishes.

Monochloracetic Acid

The Associate Referee completed collaborative studies on monochloracetic acid in carbonated beverages and fruit juices. He plans collaborative work on identification tests and further extension of the application of this method.

Dichloracetic Acid

The Associate Referee reported that the lateness of his appointment combined with the volume of his normal duties made it impossible for him to submit a report.

Mold Inhibitors-propionates

The Associate Referee completed collaborative studies on the detection of added propionate in bakery products and plans further work on propionate losses in baked goods and an investigation using chromatographic technique for the identification and estimation of acetic and propionic acid in these products. If time permits he also plans to investigate other mold inhibitors.

Thiourea

The Associate Referee submitted and recommended methods for thiourea, which are given in detail in *This Journal*, **31**, 100–104 (1948), under "Changes in Methods of Analysis."

Dulcin

The Associate Referee had been working on an alkaline-ether extraction method on carbonated beverages and a qualitative test for the extracted dulcin but the Referee was unable to contact him recently and has received no report of his investigation.

Formaldehyde

The Associate Referee spent all his available time on another A.O.A.C. problem this year but hopes to begin work shortly on the development of a more modern method for the detection and determination of formaldehyde in food products and the deletion of many of the present A.O.A.C. methods.

RECOMMENDATIONS*

The Referee recommends—

(1) That work be continued on benzoate of soda and esters of benzoic acid.

(2) That the sublimation method for saccharin determination, *This Journal,* **30,** 494 (1947), be adopted as tentative. The first line to read "Transfer 200 ml of filtrate, **32.14,** to a separator and add 15 ml HCl."

(3) That studies on the determination of saccharin in baked goods be continued.

(4) That the ferricyanide method for quaternary ammonium compounds in commercial preservatives, *This Journal,* **29,** 312 (1946), be made official, first action.

(5) That the method for quaternary ammonium compounds in table sirup, *This Journal,* **29,** 325 (1946), be made official, first action.

(6) That the method for quaternary ammonium compounds in bottled beverages containing fruit juices, *This Journal* **29,** 322 (1946), be made official, first action.

(7) That the method for quaternary ammonium compounds in beer, *This Journal,* **29,** 325 (1946), be made official, first action.

(8) That further studies on quaternary ammonium compounds be conducted as outlined in the report of the Associate Referee.

(9) That the method for the determination of monochloracetic acid, *This Journal,* **27,** 199 (1944), be adopted as official, first action.

(10) That collaborative work be carried out on monochloracetic acid as outlined in the report of the Associate Referee.

(11) That a collaborative study of the method for identifying dichloracetic acid, *This Journal,* be made and/or any other studies the Associate Referee wishes to inaugurate.

(12) That the distillation procedure, described in the report of the Associate Referee on mold inhibitors-propionates, be adopted as tentative for the detection of added propionates in bakery products.

(13) That further investigations on propionates in bakery products be undertaken as described in the report of the Associate Referee.

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* For report of Subcommittee C and action of the Association, see *This Journal,* **31,** 53 (1948).

(14) That work on a method for the detection and/or estimation of thiourea in foods be continued.

(15) That studies on methods for detection and/or determination of dulcin in food be continued.

(16) That studies on present methods for the detection of formaldehyde be made in order to modernize that section of the chapter on preservatives and artificial sweeteners.

(17) That committee C take under advisement the deletion of the sections on B Napththol and Abrastol from *Methods of Analysis* if they consider the use of these compounds to be obsolete. (This recommendation is made in view of the increase in space needed by the introduction of the above recommended methods into the chapter on preservatives and artificial sweeteners.)

(18) That a Referee be appointed to develop a method for the detection and/or determination of the newly publicized sweetener, 1-propoxy 2-amino 4-nitrobenzene.

REPORT ON THIOUREA IN FOODS

By W. O. Winkler (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

Although thiourea has been used as a drug for a considerable time, its proposed use in foods, as far as the writer is aware, is of fairly recent date. Its proposed use in foods has been to serve two distinct purposes: (1) to prevent bacterial decay such as stem end rot in citrus fruits, and (2) to prevent browning (darkening) in fruits. It is possible that other uses may have escaped discovery.

REAGENTS

A number of methods exist for determining thiourea in the free state or in the presence of inorganic materials, but most of these methods are not sufficiently specific or adaptable to use in food products, nor are they sufficiently sensitive for this purpose. Probably the most sensitive and widely used types of reagent for the determination of thiourea and its derivatives are the pentacyanoferrates derived from sodium nitroprusside. The form most commonly used appears to be that developed by Grote (1) with some slight modifications (2). This is a hydrated form of pentacyanoferrate. There exists also the amino form in which the ammonia group has replaced the nitrogen group in the sodium nitrosopentacyanoferrate (sodium nitroprusside) (3).

In most of the work done by the Associate Referee, a slightly modified form of Grote's reagent has been used but the amino form is also being investigated. Although this reagent, as well as the amino form, gives reactions with a great many different compounds, the conditions of reaction, and the colored products differ greatly. In dilute acid solution, the number of compounds giving a blue chromogen, similar to thiourea and its derivatives, is decidedly limited. Under these conditions (*p*H 3.5 to 6.8) the reaction can be made quite specific for these compounds by measuring the chromogen at the proper wave length and insuring a low concentration of other materials which produce a similar color. Thiourea produces a much more intense blue color in minute quantities than is given by any other substances known to the writer. This makes possible the diluting out of other substances, if present. The reaction appears to be a gentle oxidation followed by coupling. It is rather sensitive to the environment and is affected by a considerable number of materials. The reagent (essentially that of Grote) (1) is also sensitive and its effectiveness is quite markedly affected by the manner of its preparation. Identical conditions should always be followed, therefore, in its preparation.

The amino form of the reagent has not received as much attention, but it would appear that its preparation can be more easily controlled than that of Grote (hydrated form). It is being used at present as a qualitative test for the chemical in orange juice.

METHODS FOR FOODS

A study has been made of the application of the reaction to two foods, oranges (or orange juice) and frozen dessert peaches. Some difficulty was encountered in applying the reaction to oranges. They contain an inhibitory substance which prevents the formation of the chromogen. The interfering substance is, in the Associate Referee's opinion, doubtless a reducing substance. In order to make a quantitative determination, the Associate Referee has resorted to extraction of the thiourea with an immiscible solvent to separate the chemical from the interference. Although determinations have been made using clarifying reagents, the removal of the interference depends upon its occlusion with precipitates of other material. No clarifying agent that gives a precipitate with the interference and is safe to use with thiourea has been found. Removal of the interference by occlusion with a precipitate often carries with it some of the thiourea and such methods are therefore not deemed satisfactory.

Another possibility is the oxidation of the interference at the same time oxidizing the thiourea only to the disulfide stage which also gives a reaction with the pentacyanoferrates. Qualitative tests for presence of thiourea, based on this principle, are given in detail in *This Journal,* **31,** 104 (1948).

A study is being made to see if these tests can be made quantitative and thereby eliminate the necessity of extraction, but as yet this has not been accomplished.

THIOUREA IN ORANGE JUICE

A quantitative method for thiourea has also been proposed, and details of the procedure are published in *This Journal*, **31**, 100 (1948) under "Changes in Methods of Analysis."

FROZEN DESSERT PEACHES

The other food to which thiourea has been added at times is frozen dessert peaches. As is well known, many fruits darken on being cut and exposed to the air, due to the enzyme systems in the fruit. To prevent this darkening, various substances have been added to fruits at times. The enzyme systems also attack the thiourea and precautions must be taken to prevent this during the course of the analysis. The nature of this reaction is the subject of another paper and will not be discussed here.

The method as proposed for thiourea determination in frozen dessert peaches is given in detail in *This Journal*, **31**, 102 (1948).

RECOMMENDATIONS*

It is recommended:

(1) That the qualitative test for thiourea in orange juice, using the pentacyanoammonioferrate, be adopted as tentative.

(2) That the method for quantitative determination of thiourea in orange or orange juice be adopted as tentative.

(3) That the method for determination of thiourea in frozen dessert peaches be adopted as tentative.

(4) That the work on methods of thiourea determination in foods be continued.

REFERENCES

(1) *J. Biol. Chem.*, **93**, 25 (1931).
(2) *Ibid.*, **152**, 202 (1944).
(3) *Analyst*, **71**, 562 (1946).

REPORT ON BENZOIC AND VANILLIC ACID ESTERS

By W. J. McCarthy (Food and Drug Administration, Federal Security Agency, 501 Post Office Building, Cincinnati 2, Ohio), *Associate Referee*

During the year experimental work on the recovery of ethyl vanillate from various food products was planned. However, when attempt was made to procure ethyl vanillate through the usual channels, chemical supply houses, it was learned that the production of ethyl vanillate is still in the development stage and the product is not available commercially at present.

Inquiry was made of the authors of the research work done at the Institute of Paper Chemistry, Appleton, Wisconsin, as to methods they might have used in recovery experiments. Mr. Irwin A. Pearl, research associate, in his reply said they had not studied the point directly but that recoveries of vanillic acid esters had been made from various organic materials and he described the general method used in most cases, as follows:

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* For report of Subcommittee C and action of the Association, see *This Journal*, **31**, 54 (1948).

The material is extracted with ether (or benzene) and the ether extract is evaporated. The residue is boiled with dilute alkali to hydrolyze the ester. The mixture is cooled and extracted with ether to remove the alcohol formed upon hydrolysis. The alkaline solution is acidified with dilute sulphuric acid and again extracted with ether. The ether is evaporated and the residue dried to give vanillic acid. The vanillic acid is estimated by methoxyl analysis. In most cases the vanillic acid may be determined by actual weight along with melting point.

Since ethyl vanillate is not available commercially, Mr. Pearl very kindly sent me a laboratory sample of the product for experimental use.

It is recommended* that further study be given this problem during the coming year.

REPORT ON SACCHARIN

By MARGARETHE OAKLEY (State Department of Health, Baltimore 18, Md.) *Associate Referee*

A package of unsweetened muffin mix was used to prepare cookies containing known amounts of saccharin. To 350 grams of the mix 70 mg. of saccharin were added, in order to have a finished product containing approximately 200 p.p.m. Sufficient water was added along with the saccharin solution to produce a dough of such a consistency that it could be dropped by spoonfuls onto cooky sheets. The cookies were baked at ca 230°C., allowed to air dry overnight, and weighed. Calculated on this weight, the baked material contained 226 p.p.m. of saccharin. After comminuting the cookies by passing through a meat grinder the crumbled material was stored in glass jars.

Fifty gram samples theoretically containing 11.3 mg. of insoluble saccharin were used for all determinations.

1. A Soxhlet extractor was first tried, and after a two hour extraction with petroleum benzine to remove fats, the benzine was discarded and a six hour alcohol extraction to remove the saccharin was carried out in the same apparatus. The alcohol extract was dark brown with extraneous material some of which carried over when the extract was sublimed in an endeavor to purify the saccharin. The yield was so small that this method was abandoned.

2. A mixture of the sample in water was made alkaline to bring the

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* For report of Subcommittee C and action of the Association, see *This Journal*, 31, 53 (1948).

saccharin into solution, centrifuged, and the supernatent liquid acidified before extraction with ether. Acidification threw down such a mass of extraneous solid material that a Palkin extractor was tried; however, the ether brought the solid to the surface in the form of a gummy mass which was not a suitable medium for this type of extractor. This method was finally abandoned in favor of a modification of the official general method for the preparation of solid preparations, sec. **32.14** (**c**).

3. The sample was treated with hot water, acetic acid, and lead acetate. Instead of trying to filter the huge precipitate, it was centrifuged and the supernatent liquid strained through glass wool before acidification with hydrochloric acid and extraction with ether. It was necessary to break the emulsions formed by centrifuging. The ether extract was transferred to a sublimator with warm alcohol and sublimed at 140–160° for 1½ hours at 2 mm. pressure. The results from this method seemed promising and the sublimates appeared quite free from extraneous material. However, it was decided to check the purity of the residues by sulfate determinations. These showed the sublimates to be only a little over half saccharin, and the problem was right back where it was in 1924, when M. G. Wolf [(*This Journal*, **7**, 43 (1924)] abandoned the determination of saccharin baked foods because he could not get any better than 50 per cent recovery.

The Associate Referee is willing to make one more try to determine saccharin in baked goods before giving up the problem and therefore recommends that the study be continued another year.

REPORT ON QUATERNARY AMMONIUM COMPOUNDS IN FOODS

By John B. Wilson (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

Procedures for the determination of quaternary ammonium compounds in ten different kinds of foods were published in *This Journal* about one year ago.[1] Upon his appointment the Associate Referee decided to submit to collaborative study the ferricyanide method for examination of commercial preservatives, the two procedures for fruit juice, and several of the procedures for other foods. Accordingly, eight samples were prepared and sent out to collaborators. The samples were made up as described below.

A quantity of lauryldimethylbenzylammonium chloride which had been analyzed by the ferricyanide method and found to contain about 92 per cent of the anhydrous compound was used to make up the samples.

1. For the sample similar to commercial preservative, 25 grams of the

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[1] *This Journal*, **29**, 311 (1946).

salt was dissolved in water and made up to one liter, giving a concentration of 2.3 grams per 100 ml.

2. A quantity of table sirup containing about 15% maple sirup and 85% sugar sirup was purchased. 80 mg of preservative, corresponding to 73.6 mg of anhydrous quaternary ammonium salt was dissolved in some of the sirup and thoroughly mixed until there was a total of 1600 grams, corresponding to 4.6 mg. per 100 grams.

3. A sample of orange juice was prepared from canned orange juice by dissolving 0.16 gram, corresponding to 0.1472 gram of the anhydrous quaternary and made to four liters, which corresponds to 3.68 mg per 100 ml. of juice. After mixing as well as possible, 400 ml of juice was measured into each pint bottle, loosely stoppered with a rubber stopper, and heated in the steam sterilizer for 30 minutes and when cool the stoppers were driven in. Sterilized glassware was used throughout, but in spite of these precautions mold grew in some of the samples.

4. For the orange beverage containing about 15 per cent of fruit juice, a case of commercial still beverage was purchased. The bottles were opened and the contents transferred to a sterilized two-gallon bottle. 300 mg of the quaternary compound, corresponding to 276 mg of lauryldimethylbenzylammonium chloride, was dissolved in a few ml of H_2O added to the beverage mixed in a total volume of 5 liters. The beverage now containing 5.52 mg of the quaternary compound per 100 ml, was filled into the original bottles which were loosely stoppered with rubber stoppers and heated in a steam sterilizer for 30 minutes; after gradual cooling, the stoppers were driven in.

5. A case of imitation grape soda was purchased, the several bottles opened and transferred to a sterilized two-gallon bottle. 100 mg preservative, corresponding to 92 mg of lauryldimethylbenzylammonium chloride was dissolved in a portion of the beverage, then mixed with the main body of liquid which was made up to 5 liters, filled into the original bottles, and recapped. Since this beverage was supposed to contain no fruit juice, and was carbonated, it was unnecessary to sterilize it, although sterilized glassware was used in making up the sample to prevent a heavy contamination which might have proved troublesome. None of these samples spoiled in any way. They contained 1.84 mg lauryldimethylbenzylammonium chloride per 100 ml.

6. Sample 6 was designed to show the efficacy of the method in detecting quaternary ammonium compounds in milk accidentally contaminated by improper rinsing of equipment which has been cleansed with these compounds. A water solution containing 0.23 mg of lauryldimethylbenzylammonium chloride per 1 ml was to be mixed with milk by the collaborator, at the rate of 5 ml per 500 ml of mixture.

7. Sample 7 was designed to show the efficacy of the method for milk when the quaternary compound was added as a preservative. The water

solution containing 2.07 g of lauryldimethylbenzylammonium chloride in 500 ml was to be diluted as under (6) yielding milk containing 4.14 mg of quaternary compound per 100 ml.

8. A case of beer was purchased and the bottles cooled in ice water for several hours. A solution of 300 mg of the preservative was dissolved in water and made to 50 ml in a volumetric flask. When cold, each bottle was opened separately, 2 ml of preservative solution was pipetted into it, the bottle recapped immediately, and mixed thoroughly. In all cases only a slight head formed when the original cap was removed. In no case did a bottle foam over. This procedure yielded a beer containing 11.04 mg lauryldimethylbenzylammonium chloride per bottle or 3.11 mg per 100 ml provided the bottle contained 12 fluid ounces (355 ml).

Samples were sent to the collaborators listed below, for whose assistance we wish to express grateful appreciation.

V. D.—Halver C. Van Dame, Food & Drug Administration, Cincinnati, Ohio.
J.—Curtis R. Joiner, Food and Drug Administration, St. Louis, Mo.
We—Louis C. Weiss, Food & Drug Administration, Los Angeles, Cal.
K—Gardner Kirsten, Food & Drug Administration, New York, N. Y.
M—Dorothy Montgomery, Food & Drug Administration, Washington D.C.
W—John B. Wilson, Food & Drug Administration, Washington D. C.

The results obtained by collaborators are given in Table 1. Unfortunately, the sample of orange juice became moldy and several of the collaborators did not analyze it.

The results reported in Table 1 indicate that the "Ferricyanide Method for Quaternary Ammonium Compounds in Commercial Preservatives" should be recommended for adoption, without further work, as official, first action.[2]

The results obtained in analyzing sirup (average found, 4.48 mg/100 ml, recovery 97%); orangeade (average found, 4.97 mg/100 ml, recovery 90%), and beer (average found, 2.73 mg/100 ml, recovery 88%) warrant the adoption of these methods as official, first action.

More collaborative work should be done on the following food products: Orange juice, grape soda, and milk (preservative quantities).

Evidently an error was made in the preparation of the sample of orange juice, since collaborators found more than was to be added. In previous work in no case was a recovery of 100 per cent obtained. The results are therefore being rejected with a view to repeating the work next year.

Only two collaborators obtained good results on milk sample (No. 7). It is hoped that further experience will enable collaborators to obtain better results on next year's samples.

Since all collaborators found some quaternary ammonium compound in the "accidentally contaminated milk" containing about 2 p.p.m. of

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[2] Details of the Method are published in *This Journal*, 31, 105 (1948).

TABLE 1.—*Quaternary ammonium compounds in foods*

NO.	KIND OF FOOD	ADDED G/100 ML	FOUND					
			W	M	J	V.D.	K	Wa
					g/100 ml			
1	Preservative	2.300	2.235	2.205	2.264	2.228	2.218	2.266
			2.245	2.201	2.266	2.231	2.215	2.266
		mg/100 ml			mg/100 ml			
2	Sirup	4.6	4.3	4.4	4.6	5.0	4.7	3.8
			4.3	4.4	4.6	5.0	4.6	
			4.2					
			4.4					
3	Orange Juice	3.7[a]	4.5	3.8	3.8	[d]		4.8
			4.2	3.8	3.9			5.2
		[b]	4.0	2.2	4.3			4.1
			3.6	2.2	4.4			4.2
4	Orangeade	5.5	5.0	4.6	4.9	5.2	5.4	4.7
			5.3	4.8		5.1	5.3	4.5
			5.2[c]					
			5.4[c]					
5	Grape Soda	1.8	1.8	1.6	1.6	1.7	1.2	1.3
			1.7	1.6	1.6	1.7	1.2	1.3
			1.9					
6	Milk	0.23	0.04	0.10	0.06	0.09	0.05	0.08
			0.05	0.10	0.05	0.12	0.05	0.08
			0.04		0.08			
7	Milk	4.1	3.9	3.2	0.8	3.9	1.5	2.9
			3.9	3.3	1.0	3.9	1.4	2.7
					2.7			
					1.8			
8	Beer	3.1	2.7	2.5	3.2	2.3	2.9	3.1
			2.6	2.5		2.4	3.0	3.1
			2.5					

[a] By method for fruit juices (29, 318, 1946).
[b] By shorter method for fruit juices (29, 319, 1946.)
[c] Liquid phase was also steam distilled.
[d] Sample had spoiled.

lauryldimethylbenzylammonium chloride, further work on amounts in the category will not be done at this time.

In the method for sodas the volume of bromophenol blue reagent used (3 ml = 1.2 mg) probably does not furnish a sufficient excess of reagent to insure complete extraction of the quaternary compound. In next year's study 5 to 10 ml of the reagent will be commended, with the hope that the low results obtained by some collaborators will be avoided.

RECOMMENDATIONS*

It is recommended:

(1) That the ferricyanide method for quaternary ammonium compounds in commercial preservatives. *This Journal*. **29**, 312 (1946), be made official, first action.

(2) That the method for table sirup, *This Journal* **29**, 325 (1946), be made official, first action.

(3) That the method for bottled beverages containing fruit juice, *This Journal*, **29**, 322 (1946), be made official, first action.

(4) That the method for beer, *This Journal*, **29**, 325 (1946), be made official, first action.

(5) That collaborative study be continued on the following methods:
  (a) Method for fruit juices (*This Journal*, **29**, 318).
  (b) Shorter method for fruit juices (*This Journal*, **29**, 319).
  (c) Method for bottled sodas (*This Journal*, **29**, 323), subject to increasing the volume of bromophenol blue reagent to 5–10 ml.
  (d) Method for milk (*This Journal*, **29**, 324), on samples containing preservative quantities of quaternary ammonium compounds.

(6) That collaborative study be made of the methods for mayonnaise, salad dressings, sandwich spreads, pickles, and relishes.

REPORT ON MONOCHLOROACETIC ACID

By JOHN B. WILSON (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

For the collaborative work on monochloroacetic acid, four samples were submitted to the nine collaborators listed, with the instructions given below. Samples 1, 2, and 3 were water solutions, whereas sample 4 consisted of orange juice which was heated to 100°C in an "Arnold Sterilizer" for 30 minutes after bottling.

COLLABORATORS

G—E. H. Grant, Food and Drug Administration, Boston Mass.
Ma—Alex P. Mathers, Alcohol Tax Unit, Washington, D. C.
Me—H. J. Meuron, Food and Drug Administration, San Francisco, Calif.
Mo—Dorothy Montgomery, Food and Drug Administration, Washington, D. C.
N—E. K. Nealon, Alcohol Tax Unit, Detroit, Mich.
O—Harold F. O'Keefe, Food and Drug Administration, Chicago, Ill.
S—Angus J. Shingler, Food and Drug Administration, Atlanta, Ga.

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* For report of Subcommittee C and action of the Association, see *This Journal*, **31**, 54 (1948).

T—John A. Thomas, Food and Drug Administration, New Orleans, La.
W—John B. Wilson, Food and Drug Administration, Washington, D. C.

DIRECTIONS FOR COLLABORATORS

(For analysis of samples containing monochloroacetic acid)

Samples 1–4 inclusive:

A. Determine monochloroacetic acid in 100 ml by the quantitative method (*This Journal*, **27**, 199, 1944), in duplicate.

Note: In the titration of the excess $AgNO_3$ with NH_4CNS it has been found that the end point is more easily observed if the precipitate is removed just before the final pink color appears. To accomplish this, add the NH_4CNS soln carefully until the pink color formed by such addition fades slowly on mixing the soln. When this point is reached, shake the soln for ½ min. and filter through a folded filter into another flask. When the first flask is empty, wash down the sides with about 50 ml of water and add this to the filter after the soln has all gone through. When the wash water has passed through, complete the titration.

B. Make qualitative tests as follows:

Extract 4 separate 175 ml portions of each sample in automatic extractors, adding 6 ml of H_2SO_4 to each and extracting for about 2 hours. Test the extracts as follows:

1. *Barium test.* Treat extract (1) as directed in *This Journal*, **27**, 446, 1944, beginning "Transfer the ether extract to a separatory funnel . . . Examine the crystals under the polarizing microscope."

Note: The microscopic examination (*This Journal*, **27**, 447, 1944) should be made by a district analyst familiar with the procedure, and the remaining crystals should be forwarded to the Microchemical Section for check analysis.

2. *Indigo test on barium salt.* Treat extract (2) as above except apply the indigo test to the crystals following the procedure "Indigo Test" on the mimeographed sheet (same as *This Journal*, **28**, 303, 1945).

Note: Use the entire crop of crystals if less than 0.17 g is obtained and if 50 mg or less is obtained make up in a 5 ml graduated cylinder and add only 0.3 ml. of 1 *N* H_2SO_4.

3. *Indigo test.* To extract (3) add 3 ml of anthranilic acid reagent and evaporate the ether at a low temperature. If any insoluble matter (oily or solid) separates out, filter the remaining liquid thru a small wet filter paper into a 50 ml beaker. If no insolubles come out, transfer to a 50 ml beaker. Now apply the test as under (2) beginning "Test with litmus paper, etc."

4. *Pyridine test.* Treat extract (4) by the method (*This Journal*, **29**, 104, 1946) under the heading "Qualitative Method Applicable to Beverages and Fruit Juices," parts (1) and (2).

The results obtained by the quantitative method are given in Table 1. In the case of sample 1 the analyses by "W" and "Mo" were made within a few days after the preparation of the sample. As several collaborators mentioned that a growth of mold was found in the samples, "W" repeated the determination about 6 months later and found about 1 mg monochloroacetic acid per 100 ml. It appears, therefore, that the mold had been growing upon the monochloroacetic acid, which accounts for the low results obtained by the remaining collaborators. From a quantitative viewpoint these determinations, except those made by "W" and "Mo" soon after preparation, should be disregarded. They have been reported in the table because of their bearing upon the qualitative tests.

"G" and "S" found their continuous extractors did not take out all of the monochloroacetic acid in 2–3 hours, as recommended in the method. They were advised to test the efficiency of these extractions using a solution of monochloroacetic acid containing 1 g per liter. Both found that 5 hours were required. "G" obtained the following recoveries with 5 hours extraction:

TABLE 1.—*Monochloroacetic acid (quantitative method)*

SAMPLE	ADDED MG/100 ML	FOUND—MG/100 ML								
		W	Mo	S	G	O	T	Me	Ma	N
1	8.0	7.2	7.2	2.2	3.2	2.4	5.0	3.2	3.4	3.5
		7.7	7.0	2.2	3.4	2.8	5.0	3.0		
		7.8								
		7.7								
2	22.0	21.2	20.1	18.8	19.8	22.5	21.8	20.5	20.8	20.0
		21.2	20.5	21.3	20.3	21.7	21.9	20.8		
		21.3								
3	46.0	44.9	43.0	42.7	44.4	45.9	44.8	43.5	45.4	43.0
		45.4	43.7	44.4	45.8	47.1	44.3	43.2		
		45.9		44.9						
4	17.7	15.4	13.3	11.5	14.0	13.7	14.9	14.0	14.3	12.5
		15.2	13.3	10.8	14.7	13.5	13.3	14.0		
		16.0		13.8						
				11.2						
				10.9						

Present *Mg*	*Recovered* *Mg*
50.0	49.8
15.0	14.7
3.5	3.4

These collaborators would prefer to make the extractions in separatory funnels.

"G's" experiments show that when 180 ml of solution containing monochloroacetic acid is shaken with 100 ml of ether, 58 per cent of chloroacetic acid goes into the ether layer. Four such extractions would remove 96.9 per cent of the monochloroacetic acid; six would remove 99.5 per cent, and eight, 99.9 per cent.

"W" has found that if an equal volume of ether is used, about 80 per cent of the monochloroacetic acid goes into the ether layer. With this ratio, 3 extractions should yield 99.2 per cent recovery. In most cases, three extractions should suffice.

"G" made a check on sample 4 shaking out in separatory funnels and found 15.1 mg, as against 14.0 and 14.7 mg using the continuous extractor.

"S" also made a comparison between the two means of extraction as follows:

Sample	1	1	4	4
Continuous extractor 5 hours	2.2	2.2	11.2	10.9
Separatory funnels	2.4	2.2	8.5	12.6

There was some difficulty with emulsions when the orange juice was shaken in separatory funnels.

The recoveries of monochloroacetic acid are summarized in Table 2.

TABLE 2.—*Recoveries of monochloroacetic acid*

SAMPLE	2		3		4	
	mg	*per cent*	*mg*	*per cent*	*mg*	*per cent*
Maximum	22.5	102.3	47.1	102.3	16.0	94.1
Minimum	18.8	85.4	42.7	92.8	10.8	63.5
Average	20.9	95.0	44.6	97.0	13.5	79.4

The results obtained by collaborators on samples 2 and 3, together with those reported in previous publications[1] warrant the adoption of the method as "Official—First Action." From a regulatory standpoint low results such as were obtained on sample 4 work no hardship upon food manufacturers, but from a scientific standpoint they show the need for a more efficient method of extraction. Considerable objection has been registered by analysts in the Food and Drug Administration to the time consumed in the extractions, and several have expressed a desire to make extractions by hand using separatory funnels. Before recommending final action on the method, a further comparison should be made between automatic extraction and hand extraction in separatory funnels. Two suggestions have been made to improve the extraction procedure. The first is the salting out of the monochloroacetic acid, and the second the use of a more efficient extractor. Yakowitz, of our San Francisco Station, has designed a new extractor, which is described in a paper soon to be published in this *Journal*. Mathers, of the Alcohol Tax Unit, demonstrated that 113 mg out of 115.3 mg of monochloroacetic acid can be extracted in 30 minutes using a continuous extractor designed in that laboratory.

QUALITATIVE TESTS

The results of the qualitative tests are given in Table 3.

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[1] *This* Journal, **25:** 146 (1942); **27:** 195 (1944).

"G" is the only collaborator who obtained crystals of barium monochloroacetate from sample 1. To accomplish this he used carbonic acid to remove excess barium hydroxide, acidified the filtrate with a drop of 0.01 *N* acetic acid to discharge the pink phenolphthalein color formed on heating, and made the final crystallization from a small quantity of alcohol.

"O", who has had considerable experience with the indigo test, prefers to use a mixture of equal parts of sodium hydroxide and potassium hydroxide, instead of all sodium hydroxide, in the 50% alkali solution, in which case the fusion is carried out at 225°–240°C. with less danger of losing indigo through overheating. However, in the present instance he followed the prescribed procedure.

TABLE 3.—*Monochloroacetic acid* (*qualitative tests*)

TEST	SAMPLE	W	Mo	S	G	O	T	MB	MA
Barium	1	−	−	−	+	−	−	−	−
	2	+	+	+	+	+	−	−	+
	3	+	+	+	+	+	+	−	+
	4	−	−	−	−	−	−	−	−
Indigo Barium	1	+	−	−	[a]	−	+	−	
	2	+	+	−		+	+	−	
	3	+	+	+		+	+	−	
	4	+	+	+		+	+	−	
Indigo	1	+	+	−	[a]	+	+[b]	−	
	2	+	+	+		+	+	+	+
	3	+	+	+		+	+	+	+
	4	+	+	+		+	+	−	
Pyridine	1	+	+	−	+[c]	+	−	−	−
	2	+	+	+	+	+	−	+[d]	+[f]
	3	+	+	+	+	+	−	+[e]	+[g]
	4	+	+	+	+[c]	+	−	−	−

[a] Tests were not made but collaborator said "I can detect reasonably small amounts by the other tests."
[b] Indigo precipitate was not noticeable in the solution but was apparent on the filter paper.
[c] Tests tests made by C. S. Purcell, who was more experienced with the method.
[d] m.p. 157°C.
[e] m.p. 158°C.
[f] Wt. of crystals corresponds to 21.5 mg $CH_2ClCOOH$.
[g] Wt. of crystals corresponds to 46.6 mg $CH_2ClCOOH$.

The barium test is adapted for use on comparatively pure solutions when 20 mg or more per 100 ml are present. None of the collaborators obtained a positive barium test on the orange juice.

The indigo test on the ether extract yielded more positive results than when performed on the barium crystals; it seems advisable to confine the qualitative tests to the simple indigo test and the pyridine test which seem to be running neck and neck. The work on these two tests should be extended to other types of foods.

RECOMMENDATIONS*

It is recommended—

(1) That the method for the determination of monochloroacetic acid (applicable to carbonated beverages and fruit juices containing 5 to 150 mg CH_2Cl COOH in 150 ml), *This Journal*, 27, 199 (1944) with the addition under "Apparatus," of the statement, "2. Use any equally efficient means of extraction." be adopted as official, first action.

(2) That collaborative work be done to establish the advisability of permitting extractions to be made in separatory funnels as an alternate procedure.

(3) That collaborative work be done on other fruit juices and beverages.

(4) That collaborative work be done on the application of the method to other types of food.

(5) That collaborative study of the indigo test and the pyridine test be continued.

No reports were given on dichloroacetic acid or on formaldehyde.

REPORT ON MOLD INHIBITORS, PROPIONATES

By LEWIS H. MCROBERTS (Food and Drug Administration, Federal Security Agency, San Francisco, Calif.), *Associate Referee*

DETERMINATION OF PROPIONATES IN BAKERY PRODUCTS

Under provisions of section 701 of the Federal Food, Drug and Cosmetic Act, a hearing has been held and proposals issued for establishment of standards of identity for various types of bread (1). At this hearing evidence was presented indicating that the use of mold and "rope" inhibitors was a not uncommon bakery practice. The proposed standards of identity limit the quantities of preservatives (with appropriate label declaration) to the following:

Calcium or Sodium propionate
  In white bread = 0.32 part/100 parts flour
  In whole wheat bread = 0.38 part/100 parts flour
Vinegar—100 grain or equivalent = 1 pint/100 lb. flour
Sodium diacetate = 0.4 part/100 parts flour.

Whether or not the proposed standards are adopted it is desirable that methods for detection and estimation of these preservatives be available. The determination of propionic acid should serve well in the estimation of added propionates for there is no evidence that this acid is normally present in bakery products in other than calculated trace amounts. Acetic acid is normally present in amounts that would have to be taken into account in estimating the proportion of vinegar or diacetate added. The determination of both of these acids through adaptation of the volatile acid technique (2, 3, 4, 5) appeared promising. For supplementary confirmation the use of the chromatographic column (6) also offered possibilities.

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* For report of Subcommittee C, and action of the Association, see *This Journal*, 31, 52 (1948).

EXPERIMENTAL

In preliminary studies the writer applied the Official Method (**23.36** (**b**)) for volatile fatty acids in dried eggs to the analysis of samples of commercial bakery products, some of which were believed to contain added propionate; and to samples of bread prepared in the laboratory, without propionate, and with varying amounts of propionic acid added.

Some of the same samples were also analyzed by a modification of this procedure, which provided for ether extraction of the aqueous sample solution obtained after treatment with phosphotungstic acid and filtration (first step in preparation of the sample by the official method above cited). The extraction was carried out on an aliquot of the filtrate, acidified with sulfuric acid in a large Palkin extractor. Trial with pure formic, acetic, and propionic acids had indicated that such extraction would be 90–100 per cent complete in five hours. Following extraction, water was added to the extract, rendered alkaline to phenolphthalein with 1 *N* sodium hydroxide, and the ether evaporated. The aqueous phase was made to a convenient volume and an aliquot subjected to the usual distillation procedure (sec. **24.11**).

The purpose of the modification was to remove carbohydrates which, it was suspected, might break down during distillation to contribute volatile acids to the distillate. Incidental to the application of the modification certain observations gave rise to a suspicion that volatile acids, particularly acetic, might be contributed by the ether used for extraction. For that reason the ether was thoroughly washed with water just prior to use.

Results of these preliminary trials are given in Table 1. There is no indication, in these results, of any contribution of formic or propionic acids from a breakdown of carbohydrates during distillation. The implications are not entirely clear-cut in respect to acetic acid. The results may imply some derivation of acetic acid from carbohydrates and also from the use of unwashed U.S.P. ether in the extraction. As far as propionic acid is concerned the unmodified method gives results more closely in agreement with the theoretical.

In the course of routine operations a considerable number of samples of bakery products, suspected of containing added propionate, have been submitted to this laboratory for analysis. Table 2 presents the results obtained on a representative group. In some instances the batch formula customarily employed was known, although there is no assurance that such formula was actually adhered to in preparing the particular batches analyzed. The bread analyses have been converted to the terms of the batch formula on the convention that 150 lbs. of fresh product is equivalent to 100 lbs. of flour used in preparing it.

While lack of authenticity of the samples prevents any quantitative conclusions as to the percentage recovery, it is noteworthy that the analyses in most instances indicate a sodium propionate content materially lower than the batch formulas would have provided. Inasmuch as the method employed had yielded virtually complete recovery of propionic acid added to air-dry, propionate-free bread (Table 1), the results may reflect baking losses and/or losses of propionic acid during air-drying of the sample.

TABLE 1.—*Determination of volatile acids and recovery of added propionic acid by the official method for volatile acids in dried eggs, and by an ether extraction modification*

SAMPLE	OFFICIAL METHOD			"C" VALUE	ETHER EXTRACTION MODIFICATION			"C" VALUE
	FORMIC	ACETIC	PROPIONIC		FORMIC	ACETIC	PROPIONIC	
Laboratory prepared bread no added propionate	12	56	None	2.6	8	20	15	2.0
	13	34	4	2.4	9	34	6	2.3
	13	30	7	2.2				
Same—with 149 mg/100 gm added propionic acid	12	51	151	1.8	6	35	165	1.8
	14	54	151	1.8	9	37	161	1.7
Same—with 75 mg/100 gm added propionic acid	11	32	83	1.8	11	9	98	1.7
	13	39	78	1.8	10	13	97	1.7
Commercial Bread A	15	32	13	2.2	11	19	23	1.9
	16	30	13	2.2	8	21	20	2.0
Commercial Bread B[1]	14	28	83	1.8	13	57	62	2.0
Commercial Bread C[1,2]	27	67	117	1.9	25	88	87	2.0

[1] Ether used for extraction modification on these samples was not washed.

[2] Analysed fresh and results computed on the fresh basis.

To provide material for further study as well as for collaborative samples, authentic batches of bread containing known added amounts of propionate were baked in the laboratory. Details of preparation were as follows:

Ten pounds of "enriched bleached flour" was thoroughly mixed by rolling in a large container and then separated into three portions that were used in the preparation of three 3-loaf batches of bread. A commercial propionate obtained from a local

TABLE 2.—*Volatile fatty acids in commercial bakery products determined by official method for dried eggs*

PRODUCT	FORMIC MG/100 G	ACETIC MG/100 G	PROPIONIC MG/100 G	"C" VALUE	SODIUM PROPIONATE COMPUTED TO PARTS PER HUNDRED PARTS OF FLOUR USED	
					AS DETERMINED	FROM FORMULA
Samples air-dried and analysis for individual acids reported on air-dry basis.						
White bread —D	12	29	41	2.0	0.06	0.18
E	14	28	83	1.8	0.12	0.19
F	12	50	100	1.8	0.15	0.25
G[3]	4	43	35	2.0	0.04	0.08
H	14	56	55	2.0	0.08[2]	N. R.
Wheat bread—A	11	51	46	1.9	0.08	0.19
B	20	48	55	2.0	0.08	0.18
C	15	67	31	2.1	0.04	0.09
D	23	76	10	2.3	0.01	0.06
E[3]	10	38	57	1.8	0.09	0.06
F[3]	6	16	58	1.7	0.09	0.06
G[3]	6	40	38	2.0	0.06	0.08
Rye bread —A	11	173	44	2.2	0.06[1]	N. R.
Doughnuts —A[3]	15	None	263	1.6		N. R.
B[3]	11	None	298	1.5		N. R.
Samples analyzed fresh and analysis for individual acids reported on fresh basis.						
Cup cakes	8	19	None	2.6		N. R.
Fruit cake	72	124	54	2.2		N. R.
Light cake	36	None	138	1.6[2]		N. R.
Sweet rolls	16	31	None	2.5		N. R.
Jelly roll	23	None	74	1.6		N. R.
Chocolate cake—A	63	100	12	2.3		N. R.
B	22	55	96	1.9		N. R.

[1] Presence of propionic acid in distillate confirmed chromatographically, *This Journal*, 28, 644.
[2] Presence of propionic acid in distillate confirmed chromatographically and also by redistillation, *Ibid.*, 21, 688. [3] Analyses by J. V. Beck. N. R.—Not reported.

bakery was added to two of the portions of flour in the amounts of 0.32% and 0.16%. The following described recipe was used:

1.5 cakes yeast
1.5 tbs. sugar
1.5 pts. milk
2.5 lbs. prepared flour (±2 gms)
0.7 tbs. salt
3 tbs. shortening.

Baked at 450°F. for 15 min. and then lowered to 375°F. for 45 min.

Precautions were taken to insure that all of the 2.5 lbs. of prepared flour were incorporated in the bread. The three batches of bread designated as "A," "B," and "C" were allowed to cool for one hour prior to weighing. After 24 hours the loaves were then sliced and air dried for about 48 hours, re-weighed, crushed and ground to pass 20 mesh. The following data and calculations were recorded:

Bread Batch	"A"	"B"	"C"
Propionate—% in flour	0.32	0.16	None
Wt. (1 hr. after baking) oz.	56	56	58
Wt. (24 hrs. after baking) oz.	55.32	56.06	54.21
Ratio: Bread/Flour	1.38	1.40	1.36
Wt. Air dried, oz.	42.6	43.0	42.7
Ratio: Air Dry/Fresh	.7701	.7607	.7868

Assuming no baking or air drying loss, batches "A" and "B" (air dry) were then calculated to contain 0.30 per cent and 0.15 per cent of propionate, or 0.230 per cent and 0.115 per cent propionic acid, respectively.

The propionate, the flour and the mixtures of propionate and flour were analyzed for propionic acid.

Five grams of the propionate was made to 500 ml, and a 50 ml aliquot, acidified with sulfuric acid and diluted to 150 ml, was subjected to the regular distillation procedure (sec. **24.11**). The average of two determinations was 76.2 per cent of propionic acid, equivalent to 99.1 per cent of sodium propionate.

The flour and the flour-propionate mixtures were analyzed by the procedure for dried eggs, with results as follows:

Flour

SAMPLE	VOLATILE ACIDS (MG/100 G)			EQUIVALENT SODIUM PROPIONATE	
	FORMIC	ACETIC	PROPIONIC	*mg/100 g*	*per cent*
Flour Mix A	4.4	None	265	344	0.34
Flour Mix B	3.7	None	136	177	0.18
Flour Mix C	4.3	None	3	4	0.00

The bread samples were analyzed by four different procedures.

(1) The official method for dried eggs.

(2) The ether extraction modification described above.

(3) A preliminary dispersion of the sample in 100 ml of water, acidified with 25 ml 1 *N* sulfuric acid by means of a Waring Blendor. Modification (4) was then applied. The purpose of this preliminary treatment was to test whether low results might be due to occlusion of propionate in the bread particles, rendering the initial aqueous extraction incomplete.

(4) The detailed procedure given below and issued to collaborators for trial. This modification retains the basic principles of the official procedure for dried eggs, varying the technique of sample preparation solely for reasons of convenience in handling samples of bakery products.

TABLE 3.—*Volatile acids recovered from propionate-containing bread by four modifications of the official method for dried eggs*

(Results expressed as mg/100g air-dry sample.)

MODIFICATION	BREAD A			BREAD B		
	FORMIC	ACETIC	PROPIONIC	FORMIC	ACETIC	PROPIONIC
1	23	36	172	13	5	99
2	15	32	166	7	28	87
3	21	33	165	14	8	97
4	21 22	29 20	172 180	13 13	23 24	83 84
Propionic acid added air-dry basis			230			115

Results obtained by the four modifications are given in Table 3.

The results are in harmony with previous conclusions in regard to the absence of any advantage in the ether extraction (modification 2) and they fail to demonstrate any advantage in the more vigorous disintegra-

TABLE 4.—*Collaborative assay of sodium propionate*

COLLABORATOR*	ASSAY		AVE. "C" VALUE DETERMINED	STD. "C" VALUE PROPIONIC ACID
	PER CENT	AVE.		
Lewis H. McRoberts—Assoc. Ref., San Francisco	99.7, 98.5	99.1	1.61	1.58
William Horwitz—Minneapolis	96.7, 99.2, 98.3, 100.4, 99.4, 98.6	98.8	1.59	1.58
V. E. Munsey—Food Div., Washington, D.C.	99.2, 99.2	99.2	1.61	1.69
H. M. Bollinger—Los Angeles	98.5, 98.6	98.6	1.61	1.63
Mrs. D. W. Montgomery—Food Div., Washington, D.C.	99.4, 99.4	99.4	1.63	1.63
H. O. Fallscheer—Seattle	98.7, 98.9	98.8	1.69	1.64
H. I. Maycomber—Baltimore	99.6, 99.7	99.7	1.60	1.61
Meyer Matluck—Boston	98.5, 98.9	98.7	1.59	1.57
Average		99.0	1.62	1.62

* All collaborators are with the U. S. Food and Drug Administration, stationed at the laboratories indicated.

TABLE 5.—*Collaborative results—volatile acids in air-dried bread*
(Results expressed as mg/100 g air-dry sample)

COLLABORATOR	BREAD A—230 MG PROPIONIC ACID ADDED PER 100 G AIR DRY SAMPLE					BREAD B—115 MG PROPIONIC ACID ADDED PER 100 G AIR DRY SAMPLE					BREAD C—NO ADDED PROPIONIC ACID					STANDARD C VALUES		
				AVE C VALUE					AVE. C VALUE					AVE. C VALUE				
	FORMIC	ACETIC	PRO-PIONIC	DET'D.	COR-REC. FOR FOR-MIC	FORMIC	ACETIC	PRO-PIONIC	DET'D.	COR-REC. FOR FOR-MIC	FORMIC	ACETIC	PRO-PIONIC	DET'D.	COR-REC. FOR FOR-MIC	FORMIC	ACETIC	PRO-PIONIC
L. H. McR.	21 22	29 20	172 180	1.75	1.67	13 13	23 24	83 84	1 79	1.71	13	26	4	2.43	2.20	2.97	2.44	1.58
W. H.	19 15 16 16 15	63 31 45 42 49	141 171 159 151 159	1.80	1.72	10 9 8	37 41 38	74 72 76	1.87	1.85	8 7 6	34 28 30	None None None	2.54	2.45	3.10	2.48	1.58
V. E. M.	24 23	79 61	150 166	1.87	1.90	14 15	None None	133 133	1.64	1 55	12 11	41 46	None None	2.60	2.50	3.03	2.40	1.69
H. M. B.	17 17	29 36	177 177	1.79	1.73	9 9	20 20	93 93	1.76	1.73	7 7	25 25	1 2	2.53	2.42	3.07	2.48	1.63
D. W. M.	25 25	29 32	189 185	1.78	1 69	18 18	27 30	93 88	1.86	1.76	13 14	24 27	11 10	2.32	2.13	3.11	2.45	1.63
H. O. F.	24 25	30 36	176 176	1.78	1.69	14 17	12 7	95 98	1.80	1.70	16 16	10 15	10 6	2.42	2.09	3.10	2.49	1.64
H. I. M.	N.R.	25 27	187 187	N.R.	1.68	N R.	21 32	91 83	N R.	1.75	N R.	5 6	None None	N.R.	2.65	2.97	2.39	1.61
M. M.	26 29	11 32	192 162	1.74	1 64	18 19	26 9	84 92	1.80	1.68	16 15	16 18	9 6	2.36	2.09	2.99	2.41	1.57
Average propionic det'd.			175					93					4					
Apparent baking and air-drying loss (per cent)			24					19										

N. R. Not reported.

tion treatment constituting modification 3. The fourth modification gives entirely comparable results and was selected for collaborative study because of its greater convenience. Its details are given in the preceding number of *This Journal*, **31**, 99 (1948).

COLLABORATIVE STUDY

The Associate Referee has been fortunate in receiving the cooperation of seven collaborators, representing six different laboratories. Each collaborator was provided with subdivisions of air dried breads, A, B, and C, described above, and a portion of propionate used in their preparation. They were advised as to the results obtained by the Associate Referee in assay of the propionate sample, and were requested to determine and report its calculated sodium propionate content as a check on the standardization of their volatile acid apparatus.

Results are given in Table 4.

The collaborators were not advised of the amounts of propionic acid added to the collaborative bread samples. They were requested to determine the volatile acids by the procedure described above, a copy of which was provided, and to report the data presented in Table 5.

It was also suggested that the collaborators apply the chromatographic technique (6) on distillates of the bread samples if time and facilities were available. Munsey and Montgomery report the results presented in Table 6.

TABLE 6.—*Volatile acids in air-dry bread determined by chromatographic procedure*

(Results expressed as mg/100 g air-dry sample)

ACID	BREAD A		BREAD B		BREAD C	
	V.E.M.	D.W.M.	V.E.M.	D.W.M.	V.E.M.	D.W.M.
Acetic	23	37	18	26		27
	24	38	15	27	16	29
Propionic	147	168	70	87		
	136	168	66	87	2	None

DISCUSSION

On the whole, the results reported for propionic acid are reasonably consistent and imply that the suggested distillation procedure can be relied upon to detect added propionate in amounts greater than the equivalent of 20 mg propionic acid per 100 grams of sample analyzed. The results for acetic acid and formic acid are not as consistent but nevertheless demonstrate that these acids are naturally present in bakery products in significant amounts.

The apparent total baking and/or air-drying loss (if the loss is due to these circumstances) is quite substantial. If such loss is incurred in air-drying, it can, of course, be eliminated by analyzing the fresh sample. It was essential that the method of analysis be proven reliable before investigation of this loss could be undertaken intelligently. The extent and character of the collaborative results is believed to be such as normally to support a recommendation for adoption of the procedure as a quantitative method for determination of propionic acid. Pending clarification of the apparent baking and/or drying loss, however, the method must be viewed as qualitative only, notwithstanding the consistency of the collaborative results.

ACKNOWLEDGMENT

The Associate Referee acknowledges with appreciation the assistance of the collaborators whose names are given in Table 4 and whose results are identified by their initials elsewhere.

RECOMMENDATIONS*

It is recommended:

(1) That the distillation procedure described herein be adopted as tentative for the detection of added propionate in bakery products.

(2) That investigation of the nature and cause of the loss of propionic acid, referred to herein as "apparent baking and/or air-drying loss," be undertaken.

(3) That further investigation of the chromatographic technique, for the identification and estimation of acetic and propionic acids in bakery products, be undertaken.

REFERENCES

(1) *Federal Register*, Vol. 8, 10782–3, 8/3/1943.
(2) Hillig, *et al.*, *This Journal*, **22**, 116–118 (1939).
(3) Hillig, Fred, *Ibid.*, **25**, 176–195 (1942).
(4) ——, *Ibid.*, **26**, 198 (1943).
(5) ——, *Ibid.*, **27**, 204–223; 237–240 (1944).
(6) Ramsey and Patterson, *Ibid.*, **28**, 644–656 (1945).

No report was given on diacetates.

No report was given by the General Referee on eggs and egg products.

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* For report of Subcommittee C and action of the Association, see *This Journal*, **31**, 53 (1948).

REPORT ON ADDED GLYCEROL IN EGGS

By George E. Keppel (Food and Drug Administration,
Federal Security Agency, Minneapolis, Minn.),
Associate Referee

The present quantitative method for added glycerol in eggs was adopted tentatively in 1934 with the recommendation that further studies be made. In 1939 a brief progress report described some preliminary work on separating glycerol by distillation with superheated steam. No further reports have been submitted.

A survey of the literature indicates increasing use of the Malaprade periodate oxidation method for determining glycerol in mixtures. Amerine and Dietrich (*This Journal*, **28**, p. 408, 1943) use this method for glycerol in wine, and Shupe (*Ibid.*, **28**, 249, 1943) describes periodate methods for glycerol and glycols in cosmetics. The determination is based on the oxidation of glycerol to formaldehyde and formic acid by means of periodic acid or by periodate salt. Glycerol may be calculated from the amount of acid formed, as determined by direct titration, also by measurement of the excess periodate. The oxidation proceeds at room temperature and is more selective than a dichromate oxidation. However, certain substances such as dextrose, levulose, and certain organic acids among others, react with periodate and hence must be removed.

The above method was applied to samples of whole egg clarified by the present method for glycerol in egg. Blanks were high, ranging from 0.2–0.3 per cent of apparent glycerol. In view of the results, work was diverted to the problem of isolating glycerol in products entirely free from interfering egg constituents. A number of procedures based on published methods for glycerol in various food products have been examined. Blanks as low as 0.08 per cent have been obtained, but further work will need to be done before a method can be submitted.

It is recommended* that studies on methods for determination of added glycerol be continued.

REPORT ON ACIDITY OF FAT IN EGGS

By F. J. McNall (Food and Drug Administration, Federal
Security Agency, Cincinnati, Ohio), *Associate Referee*

In accordance with the recommendation of Subcommittee C (*This Journal*, **30**, 50) methods for the determination of acidity of fat in eggs were studied.

The present official method I(1) for dried eggs is based upon the process of drying the eggs at 55°C. in a vacuum oven to constant weight, extracting the fat in a continuous extractor with anhydrous ether, and then titrating the ether extract dissolved in neutral benzene with sodium ethylate. The results are reported as ml of 0.05 *N* sodium ethylate per g of ether extract. Method II, (1) the present rapid tentative method for dried eggs is essentially the same as Method I except that the eggs are extracted with ether without preliminary drying, and instead of using a continuous extractor, repeated extractions are made of the egg material in a small flask, the clear ether layer being decanted through a filter into a weighed flask. The ether is then evaporated, the residue dried, weighed, and titrated with sodium ethylate as in Method I.

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* For report of Subcommittee C and action of the Association, see *This Journal*, **31**, 51 (1948).

Kline and Johnson (2, 3), in a recent study of lipolytic activity in stored dried eggs, have shown that a specific method for measuring glyceride hydrolysis is necessary. In this work they have shown that as much as 70 per cent of the total acidity of ether extracts of egg powders as obtained by the A.O.A.C. method is due to cephalin. The cephalin fraction of animal phospholipids acts as monobasic acid and is completely titrated in solvents of low dielectric value, with phenophthalein as indicator. They have also shown that the acidity of the ether extract increases with the increase in moisture content of the eggs since larger amounts of phospholipids are extracted by the ether at higher moisture levels.

This paper further describes a method whereby the true fatty acid content of dried eggs may be determined by extracting the fat and fatty acids with acetone. The phospholipids are only slightly soluble in acetone and may be completely removed by treatment with alcoholic magnesium chloride.

Kline and Johnson have shown that by their proposed method preliminary drying is unnecessary. Further work indicated that a possible source of error in the determination of free fatty acids in dried eggs, particularly in eggs where the acids have developed in the liquid state before drying may be due to the formation of a fatty acid-protein complex. Complete recovery of the fatty acids cannot be made unless the *p*H of the egg mixture is adjusted to 4 prior to drying. However, fatty acids which have developed during storage in the dried eggs may be completely extracted at the normal *p*H of the powder, since the medium is nonaqueous and the fatty acids have no opportunity to combine with the bases present in the egg.

The method proposed by Kline and Johnson is as follows:

Transfer 2 g egg powder to a 40 ml conical, graduated centrifuge tube and add 15 ml of acetone. Let stand for 0.5 hour with frequent stirring. Centrifuge and decant into centrifuge bottle. Repeat extraction 4 times using 10 ml portions of acetone, allowing 5–10 min. extraction time. To the combined acetone extracts, which should be kept to a volume of 60 ml, add 1 ml of a satd soln of magnesium chloride in absolute alcohol. Mix well by swirling and then centrifuge. Decant supernatant liquid into a 500 ml separatory funnel. Wash the residue twice with 5 ml portions of acetone, centrifuging after each washing; and combine washings with the original supernatant liquid in the separatory funnel.

Add 30 ml of petroleum ether to the acetone soln and thoroly mix. Add 200 ml of

H_2O and agitate contents of funnel gently to prevent emulsion formation. Allow phases to separate and discard the aqueous acetone. Wash the petroleum ether with two 25 ml portions of water and transfer the petroleum ether quantitatively to a 125 ml Erlenmeyer flask. Add 50 ml of *iso*propanol and 10 drops of 10% ethanolic phenolphthalein. Titrate the soln with 0.025 *N* sodium ethylate.

Samples of powdered egg yolk and whole egg were obtained by the Associate Referee to compare results for the acidity of fat as determined by the present A.O.A.C. methods and the proposed method of Kline and Johnson.

Results for acidity of fat

	ML NA ETHYLATE/2 G SAMPLE	
	EGG YOLK	WHOLE EGG
A.O.A.C. Method I Vacuum oven 55°C.	4.35	1.90
A.O.A.C. Method I Vacuum oven 100°C.	3.95	1.85
A.O.A.C. Method I Without drying	4.10	2.15
A.O.A.C. Method II	4.50	2.25
Proposed acetone method without drying	1.67	1.13
Proposed acetone method after drying, vacuum oven 100°C.	1.70	0.90

DISCUSSION

The above results confirm the work of Kline and Johnson that the acetone extraction method gives results for the acidity of fat which are approximately 60 per cent less than the A.O.A.C. methods, the difference being due to the presence of titratable phospholipids which are extracted by ether but are insoluble in acetone.

RECOMMENDATIONS*

It is recommended that the proposed acetone method for determining acidity of fat in eggs be further studied.

REFERENCES

(1) *Methods of Analysis, A.O.A.C.* (1945, 6th ed.), **23.30, 23.31.**
(2) Leo Kline and C. M. Johnson, *Ind. Eng. Chem., Anal. Ed.*, **18**, 35 (1946).
(3) *Ibid.*, **18**, 617 (1946).

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* For report of Subcommittee C and action of the Association, see *This Journal*, 31, 51 (1948).

REPORT ON DECOMPOSITION IN FOODS

By W. I. Patterson (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

The report on "Decomposition in Shellfish" includes collaborative data on the indole content of shrimp, oysters, and crabmeat of varying stages of decomposition. Earlier data of individual analysts on a considerable number of samples of each of the above shellfish corroborate the usefulness of indole as a criterion of decomposition in these products. The results support the recommendation that the method for indole be adopted as official, first action. The Referee also concurs in the other recommendations of the Associate Referee.

The Associate Referee's progress report on "Decomposition in Fruits" describes some of the attempts to correlate rot in apples with a chemical test. At the present stage of development, no recommendation is made toward adoption of a definite procedure, but only that further study be made on the problem in an effort to select that chemical test with the best promise of becoming useful for regulatory purposes.

It is recommended* that studies be continued on fish and dairy products.

No reports were given on decomposition in fish products or in dairy products.

REPORT ON DECOMPOSITION IN FRUIT PRODUCTS

By Thomas H. Harris† (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.), *Associate Referee*

The use of considerable amounts of rotten fruit in the manufacture of fruit juices, jellies, and butters is easily concealed because their presence cannot be detected by odor or taste. Methods in use at present for detecting rot in fruit products are (1) The Mold Count Method and (2) The Rot Fragment Count. These methods have been used with much success on fruit products that have been processed in such a way as not to remove the mold or rot fragments, but they are of little value when applied to clear jellies and juices. Other means are desirable for establishing the presence of rotten apples, when used, where present methods are inapplicable, and in other cases, to supplement the findings based on mold or rot fragment count. The purpose of the work summarized in this report was to study the composition of juice from rotten apples with the view of developing such a chemical method.

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* For report of Subcommittee C and action of the Association, see *This Journal*, 31, 51 (1948).

† Present address: Chemical Section, Insecticide Division, Livestock Branch, Production and Marketing Administration, U. S. Dept. of Agriculture, Beltsville, Md.

PREPARATION OF MATERIAL FOR STUDY

Preliminary experiments on good and rotten apple juice to ascertain qualitative chemical differences made it clear that large quantities of juice would be required in order to isolate a sufficient quantity of the components for chemical characterization.

Approximately 20 bushels of rotten apples were macerated, a filter aid was added ("supercel" was found to be suitable), and the juice was pressed out. The apples had been discarded as unfit for use. Those individual apples containing appreciable quantities of sound tissue were either not used or the sound tissue was removed. The juice from these apples will be referred to in this work as 100% rotten juice. Approximately 50 gallons of juice were obtained and immediately concentrated in vacuo to give a dark colored thick sirup with a solids content of 60 per cent. This concentrated juice was poured into 1-quart ice cream containers and stored at 0°F. for further study.

Twelve bushels of good apples (grade 2) were processed in exactly the same manner as above. The concentrated good apple juice soon solidified to a gel, but the rotten juice did not, thus indicating a partial breakdown of the pectin present.

PECTIN DEGRADATION PRODUCTS

One of the first problems undertaken in this study was the search for the presence of some product or products of pectin degradation which would be indicative of rot. The micro-organisms that invade the damaged fruit and cause it to rot have long been known to constitute a rich source of pectic enzymes, and it appeared reasonable that certain degradation products of pectin would be present in rotten apple juice, and would either be absent or present in much smaller quantities in good juice. In the case of apples, no naturally occurring pectinase activity has been reported, and in the present investigation, none could be demonstrated.

Three such products of pectin degradation were found to be present in rotten apple juice, and two of these will be mentioned only briefly, since neither appeared very promising as the basis of a method for detecting rot

Alcohol-Soluble Non-Dialyzable Substance:

This substance was obtained by precipitating a concentrated solution of rotten apple juice from 80% ethyl alcohol to remove the pectin and dialyzing the filtrate in a cellophane tube overnight against cold running tap-water. A small quantity of precipitate separated from solution during dialysis, which gave a strong positive test for pentose. A quantity of good apple juice treated similarly gave no such substance, but the quantity of the substance obtained from the rotten apple juice was too small to be of any consequence.

Alcohol-soluble Furfural-yielding Substances:

McKinnis (1) investigated the presence of free pentoses in an 80–90%

alcohol filtrate of "Delicious" apples and concluded that, if any were present, they were there only in traces. He failed to get an estimable precipitate of furfural phloroglucide but he did not indicate the weight of starting material.

Ehrlich (2) found a substance which he called araban, and which could easily be separated from pectin by virtue of its solubility in 70% alcohol. It seemed likely that as a result of enzyme action increasing quantities of free pentoses would be released in the juice, and to answer this question the following experiments were carried out:

Two hundred g of good apples were macerated and extracted with water and the extract concentrated to a weight of 65 g. To this was added with stirring 346 ml of 95% ethyl alcohol, and the precipitated pectin was removed by filtration with added filter-aid. After removal of the alcohol in the filtrate by distillation in vacuo, the solution was diluted to 250 ml in a volumetric flask. A furfural determination on a 10 ml aliquot of the solution according to the method of Duncan (3) showed the presence of 81.3 mg of pentose (calculated as arabinose) in 100 g of apples.

The same quantity of rotten apple tissue treated similarly was found to contain 219 mg of pentose per 100 g of tissue.

One of the aims in this work was to find, if possible, some substance in rotten apple juice that was absent in good juice, and for this reason no further study of the pentose content was made.

D-Galacturonic Acid:

Ehrlich (4) described a specific test for galacturonic acid in which a brick-red precipitate was obtained on heating a basic lead acetate solution of this substance. When the test was applied directly to 1 ml of 100% rotten apple juice, a faint positive test was given. A more distinct and characteristic test was obtained on a solution of the acids obtained from the juice by precipitation with lead acetate from 80% alcohol solution, after removal of the pectin. This finding looked most promising, particularly since very little if any free galacturonic acid could be detected in good apple juice. Galacturonic acid was subsequently isolated from rotten apple juice as the *p*-bromphenylhydrazone *p*-bromphenylhydrazine derivative.

A method was devised for the quantitative determination of galacturonic acid in apple juice after removal of the sugars and other interfering substances. The galacturonic acid was determined in the final solution by a modification of the method of Deichmann and Dierker, and this modified method is given in detail below.

REMOVAL OF SUGAR, PECTIN, AND OTHER INTERFERING SUBSTANCES

Concentrate 100 ml of apple juice at reduced pressure, in a 500 ml Claisen distilling flask with a water aspirator, to ca 25 g, and transfer to a weighed 400 ml beaker. (If the soln is concd much below 25 g, there is danger of the concd juice gelling, a result which makes transfer to the beaker difficult.) The concd juice, plus the water used in the transfer, must not be permitted to exceed 30 g in weight. Add 2 g of filter-aid (Celite was found to be suitable) to the 30 g of concentrate, and add

160 ml of 95% ethyl alcohol, with stirring to precipitate the pectin, and filter the mixture with suction. Wash the residue, after pressing with a glass stopper, with ca 10 ml of 80% ethyl alcohol, and discard it. Then transfer the filtrate and washings to a 400 ml beaker and precipitate the acids by adding 2 g of neutral lead acetate ($Pb(CH_3COO)_2 \cdot 3H_2O$), dissolved in ca 10 ml of warm 80% alcohol. Add 2 g of filter aid to the suspension of lead salts, and filter the mixture on a 7 cm Büchner funnel. Press the mixture of Celite and lead salts on the funnel with a flat-top glass stopper, and transfer to a 50 ml beaker. Wash the mixture twice, by stirring with a minimum of 80% alcohol, filtering with suction each time, then transfer to a 250 ml Erlenmeyer flask. Add 100 ml of distilled water, disintegrate the mixture with a stirring rod, and decompose the salts with a current of H_2S gas. Remove the filter aid and PbS by filtration with suction, and concentrate the filtrate and washings to remove the excess H_2S, and then dilute to 100 ml with distilled water in a volumetric flask.

DETERMINATION OF GALACTURONIC ACID

Weigh 50 mg of naphthoresorcinol on an analytical balance, transfer to a 25 ml glass-stoppered cylinder, and add 1 ml of the soln of acids from apple juice, 2 ml of 50% alcohol, and 2 ml of concd HCl. Shake the mixture to dissolve the reagent, and place the stoppered cylinder in a water bath at 50°C. for 90 min. Cool the mixture to room temp., add ethyl ether to the 25 ml mark, and extract the pigment by shaking vigorously for about 10 min. After standing for ca 10 min., decant the ether extract into a colorimeter tube containing a small quantity of Na_2SO_4. Stopper the tube with a cork, whirl a few times to clear up the solution; and determine the optical density at 580 mμ (a Lumetron colorimeter, Model 400 A, was used).

The galacturonic acid content of three samples of apple juice, determined according to the above procedure, is shown in Table 1.

TABLE 1.—*Galacturonic acid content of apple juice*

SAMPLE	GALACTURONIC ACID
	micrograms/ml juice
Grade 1 juice ("Red Delicious")	35
Grade 2 juice (variety composite)	81
100% rotten juice (variety composite)	1580

It was of interest to determine the influence of variety on the content of galacturonic acid in apple juice and the data are given in Table 2.

TABLE 2.—*Relationship between apple variety and galacturonic acid present*

VARIETY	GALACTURONIC ACID
	micrograms/ml juice
Red Delicious	24
York Imperial	25
Black Twig	54
Lowry-1	32
Lowry-2	38
Western Winesap	13
Grimes Golden	40
Ben Davis	20
Jonathan	19

ACCURACY OF THE METHOD

The accuracy of the above-described method will be influenced by the completeness of precipitation of the galacturonic acid with lead acetate, and by the presence of substances other than galacturonic acid in the lead acetate precipitate which may react like galacturonic acid in the final color reaction. To determine the completeness of precipitation of the galacturonic acid by lead acetate, the following experiment was carried out:

To 100 ml of 80% ethyl alcohol, containing the quantities of sugar, malic acid, and galacturonic acid shown in Table 3, was added 2 g of filter aid and 2 g of $Pb(CH_3COO)_2 \cdot 3H_2O$ dissolved in 5 ml of hot 80% alcohol. The suspension of lead salts was filtered with suction using a flat-top glass stopper for pressing the filter cake as previously described. The lead salts were washed twice by suspending in a minimum of 80% alcohol and filtering, after which they were decomposed with H_2S, filtered, concentrated to remove the excess H_2S, and diluted to 100 ml with H_2O. One ml of this solution was analyzed for galacturonic acid as previously described.

TABLE 3.—*Recovery of galacturonic acid in lead acetate precipitate*

VOL. SOLN.	SUCROSE ADDED	MALIC ACID ADDED	GALACTURONIC ACID ADDED	GALACTURONIC ACID FOUND
	g	*g*	*micrograms/ml*	
100	0	0.5	10	12
100	0	0.5	25	26
100	0	0.5	50	45
100	0	0.5	100	92
100	5	0.5	50	48
100	10	0.5	50	48

As seen in Table 3, there is practically quantitative recovery of the galacturonic acid in the precipitate. Occlusion of the lead galacturonate with the relatively large quantity of lead malate is probably an important factor in the quantitative precipitation of galacturonic acid in such small quantities.

The naphthoresorcinol reaction has frequently been criticized for lack of specificity, but the modification of Deickmann and Dierker (5) represents a great advance in improving the specificity of this reaction. A number of other acids which could conceivably be present in fruit juices were tested and the results are shown in Table 4.

GALACTURONIC ACID AS AN INDEX OF ROT IN APPLES

The enzyme galacturonase, which is a member of the pectinase group, and which liberates galacturonic acid from polygalacturonides, was found by Kertesz (6) to be present in tomatoes but absent in apples. The increase in galacturonic acid in apples on rotting is evidently due to the action of galacturonase of microbial origin.

Galacturonic acid liberated from pectin by bacterial enzymes would appear to be a satisfactory compound upon which to base a method for detecting rot in certain apple products. It is reasonably heat-stable, non-volatile, and can be determined, after removal of interfering substances, in microgram quantities. Its presence in normal fruit will place some limitations on its use as an index of rot, but does not render such a method useless.

In some cases commercial apple juice is clarified, prior to pasteurization and filtration, by certain enzyme preparations which are known to liberate galacturonic acid from the pectin present. Obviously a method based upon galacturonic acid would be of no use on such a product, but could be applied to such products as apple butter, jelly, and juice not clarified by enzymes.

TABLE 4.—*Color given by various acids in the naphthoresorcinol reaction*

ACID	CONCENTRATION	OPTICAL DENSITY
	micrograms/ml	*580 mu*
Galacturonic	50	0.20
Pyruvic	1,000	.04
1-Ascorbic	250	.01
Oxidized ascorbic[1]	250	.05
Levulinic[2]	250	.05
2-Ketogluconic	250	.01
5-Ketogluconic	250	.01
Gluconic	250	.01
Citric	250	.01
Malic	10,000	.01
Tartaric	250	.01
Reductinic	250	.01

[1] Oxidized by aeration.
[2] Impure product.

It is felt that the present method is a step forward in the solution of the difficult problem of rot in fruit products, and particularly as applied to apples. Some work remains to be done to thoroughly test the usefulness of the method.

RECOMMENDATIONS*

It is recommended:

(1) That the proposed method for galacturonic acid in apple products be studied further in order to more definitely define its potential usefulness for detecting rot in apple products;

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* For report of Subcommittee C and action of the Association, see *This Journal*, **31**, 51 (1948).

(2) That substances other than galacturonic acid be investigated as indices of the use of rotten apples in the manufacture of apple products.

REFERENCES

(1) McKinnis, R. B., *J. Am. Chem. Soc.*, **50**, 1911 (1928).
(2) Ehrlich, F., and Haensel, R., *Cellulosechemie*, **17**, 1–12, 13–16 (1936).
(3) Duncan, I. J., *Ind. and Eng. Chem., Anal. Ed.*, **15**, 162 (1943).
(4) Ehrlich, F., *Ber.*, **65**, 352 (1932).
(5) Deichman, W. B., and Dierker, M., *J. Biol. Chem.*, **163**, 753 (1946).
(6) Kertesz, Z. I., *Food Research*, **3**, 481 (1938).

REPORT ON DECOMPOSITION IN SHELLFISH

INDOLE IN SHRIMP, OYSTERS, AND CRABMEAT

By R. E. Duggan (Food and Drug Administration, Federal Security Agency, New Orleans, La.), *Associate Referee*

W. I. Patterson[1] has recently reviewed the general subject of the chemical detection of decomposition in foods; therefore there is no need for a review of those fundamental principles in their application to the specific problem of decomposition in shellfish.

Shrimp, oysters, and crabs form the major commercial catch of shellfish. The commercial catch of clams, lobster, and crayfish is much smaller. The search for methods, other than organoleptic, for the detection of decomposition in these products has been in progress for a number of years.

King, Flynn, and Gowanloch[2] proposed the use of the indole content of canned oysters to detect the use of decomposed raw stock. They found that fresh oysters contain little or no indole, and that the indole content of oysters allowed to spoil before canning is much greater. A small amount of some substance reacting as indole to p-dimethylamino-benzaldehyde was found in fresh oysters and evidence was presented indicating that this substance was not indole. Beacham[3] substantiated the work of King, Flynn, and Gowanloch on oysters in an investigation of the decomposition of oysters and clams. Beacham concluded that indole could be used as an index of decomposition in oysters and clams. He also reported that small amounts of volatile acids were found in fresh clams and that substantially larger amounts of volatile acids were present in decomposed clams. In addition to confirming the conclusion of King, Flynn, and Gowanloch concerning the presence of indole-reacting substance (not indole) in fresh oysters, Beacham reported that larger quantities of a similar substance were found in fresh clams.

Duggan and Strasburger[4] investigated the relationship of indole and organoleptic decomposition in shrimp. No indole was found in fresh shrimp. Large amounts of indole were found in decomposed shrimp and correlated with the organoleptic degree of decomposition. They reported that different types of decomposition produced varying amounts of indole. The decomposition characterized as ammoniacal produced smaller amounts of indole than was found in the putrefactive type of decomposition. It was also found that some lots of decomposed shrimp contained insignificant quantities of indole.

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[1] *This Journal*, 28, 233 (1945).
[2] *Ibid.*, 28, 385 (1945).
[3] *Ibid.*, 29, 89 (1946).
[4] *Ibid.*, 29, 177 (1946).

An investigation of the significance of indole in crabmeat is being carried out at the present time by the U. S. Food and Drug Administration. The results of this investigation are not available at this time, and the value of indole as an index of decomposition in crabmeat has yet to be evaluated. However, since collaborative determinations of indole in oysters and shrimp were being undertaken, it was decided to extend the study to include crabmeat. Very little additional expense was incurred in the collection of this information on crabmeat. The same determinations, made later on as a separate project, would have involved a much larger outlay of time and money.

The basic method for the determination of indole in the above reports was the distillation procedure of Clarke, *et al.*[5] They reported that the method had been successfully used by five chemists in two different laboratories. No collaborative work was reported in connection with the investigations of indole in shellfish. Since indole has been shown to be significant in the detection of decomposition in shellfish[2,3,4] it was felt that the collaborative study of the method was indicated. The methods studied were those used by King, Flynn, and Gowanloch, Beacham, and Duggan and Strasburger, in their respective investigations. The procedure used in the collaborative work incorporated the worthy contributions of each of the above investigators to the basic procedure of Clarke, *et al.*

Several unsuccessful attempts were made by the Associate Referee to eliminate the substance in fresh oysters which reacts like indole. Although the error involved is small, it is advisable to eliminate such interferences where possible, and additional attempts will be made to eliminate this substance without the loss of significant quantities of indole.

COLLABORATIVE STUDY

Samples of shrimp, oysters, and crabmeat were selected in order to approximate the range of indole contents which might be encountered in commercial samples. The collaborative work on shrimp was performed approximately a year in advance of the determinations on oysters and crabmeat. Since satisfactory results were obtained by the collaborators on the recovery of indole added to shrimp, it was not considered necessary to repeat recovery experiments on oysters and crabmeat.

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[5] *Ibid.*, 20, 475 (1937).

The collaborative sample of each product consisted of a portion of a finely ground, well mixed sample. The different portions were frozen and shipped under dry ice refrigeration to the participating laboratories. Drained meats of canned oysters and canned crabmeat were used as samples of these products. The samples of shrimp were prepared from raw stock. Details of the method are given, under "Changes in Methods of Analysis," *This Journal*, **31**, 96 (1948). Results are shown in the table on the next page.

DISCUSSION OF RESULTS

The tabulation presents the results obtained by nine chemists in seven laboratories in different cities. An inspection of the results shows that the greatest variations were encountered in the shrimp samples. Undoubtedly some of the differences were due to sampling error, and others were probably due to the inexperience of the chemists in the use of the method. The differences were not considered of sufficient magnitude to warrant repeating the experiment. In connection with the determination of indole in shrimp, two collaborators[6] reported that traces of chlorine in the distilled water were responsible for the partial or complete destruction of indole in the determination. They suspected that traces of this element in ordinary distilled water was very common. The Associate Referee also found that indole was destroyed by traces of bromine.

The results obtained by the collaborators on the samples of oysters and crabmeat are excellent.

It is apparent that duplicable results can be obtained by different chemists having a minimum of experience with the method.

RECOMMENDATIONS[*]

It is recommended—

(1) That the method presented for the determination of indole in shellfish be adopted as tentative.

(2) That the investigation of methods to eliminate the indole reacting substance found in fresh oysters be continued.

(3) That substances other than indole be investigated as indices of decomposition in those types of decomposition which form little or no indole.

ACKNOWLEDGMENTS

The Associate Referee wishes to acknowledge his indebtedness to D. Banes, Chicago Station; H. W. Conroy and H. P. Bennett, Kansas City Station; Dona Clarke, Minneapolis Station; M. L. Dow, St. Louis Station; L. La Grange, New Orleans Station; H. Reynolds, Food Division; and H. C. Van Dame, Cincinnati Station, for their splendid cooperation and interest in this collaborative study.

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[6] Messrs. Bennett and Conroy, Kansas City Station, U. S. Food and Drug Administration.

[*] For report of Subcommittee C and action of the Association, see *This Journal*, **31**, 51 (1948).

Collaborative results—indole in shrimps, oysters, and crabmeat

LABORATORY	CHEMIST	RECOVERY INDOLE ADDED TO SHRIMP	INDOLE—MICROGRAMS/100 G										
			SHRIMP, SAMPLE NO.					OYSTERS, SAMPLE NO.			CRABMEAT, SAMPLE NO.		
			1	2	3	4	5	1	2	3	1	2	3
		per cent											
A	A	88.7	0	17.8	787	1.1	91	5.0	16.0	23.0	13.0	68	180
		93.3	0	17.3	774	1.0	92	5.0	16.0	22.0	15.0	66	160
		95.3											
		90.7											
B	B	97.5	1.2	20.0	1000	0	106	2.2	15.0	24.8	11.3	64	189
		100	1.5	20.2	1000	0	106	2.2	15.0	27.2	10.6	64	190
		101											
		101											
C	C	—	0	21.0	920	3.0	101	2.5	15.0	21.5	11.2	58	181
	D	—	0	19.4	916	3.0	100	2.4	15.0	22.7	11.2	60	184
D	E	97	0	17.4	1249	4.8	98	0.4	16.0	22.8	8.8	57	208
		105	0	17.6	1250	4.0	99		14.2	22.6	9.6	57	212
		97											
E	F	101	1.2	10.6	830	6.0	110						
		86	1.6	9.0	870	4.2	111						
		93											
	G	—	0	18.6	960	3.2	99	2.2	13.6	20.0	11.6	65	208
			0	17.6	904	2.0	96	2.2	13.6	22.8	11.0	68	207
F	H	97	0	5.6	820	2.4	101						
		98	0	6.2	767	1.2	101						
G	I	—						2.4	16.2	24.8	11.4	67	198
								2.5	16.2	25.4		67	202
Average			0.4	15.6	932	2.6	101	2.6	15.2	23.3	11.3	63	193

REPORT ON GELATIN, DESSERT PREPARATIONS, AND MIXES

By S. C. Rowe (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

The Associate Referee has submitted a report recommending that changes be made in paragraph **9.6** (p. 104, *Methods of Analysis*, 6th Ed.), for determining the jelly strength of plain gelatine.

Your General Referee recommends* that the changes in the method as outlined in the report of the Associate Referee be made and that the revised method be adopted by the Association as tentative. It is further recommended that this new revised method under paragraph **9.6,** for the determination of jelly strength of plain gelatin, be studied collaboratively with a view to making the method official.

Another method which needs further study is the method for jelly strength of gelatin dessert powders under paragraph **9.12.** This method deals solely with a determination of the jelly strength of the finished dessert powder and is not a method for the determination of the amount of gelatin it contains. It is recommended that the principles of the method for determining the jelly strength of plain gelatin be given consideration in a study of the method for the jelly strength of gelatin dessert powders as set forth under paragraph **9.12,** and that the method be studied collaboratively with a view to making it official.

REPORT ON JELLY STRENGTH

By Paul A. Kind (Kind and Knox Gelatin Company, Camden, N. J.), *Associate Referee*

Under the heading **9.6,** Jelly Strength (gelatine—tentative), there is given a method for the determination of jelly strength which is essentially the same as the method adopted and used for over twenty years by the Edible Gelatin Research Society of America, Inc. However, this method has one extremely significant variation, namely that the concentration of gelatine under test is set at 7.5 gm of sample in 100 ml of water, instead of the 7.5 gm of sample in 105 gm of water as employed by the Gelatin Association. This is a very serious variance. Its magnitude is illustrated by the following determinations with gelatines of various types.

7.5 gm of gelatine 105 gm of water	*7.5 gm of gelatine 100 gm of water*
135	148
172	182
175	182

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* For report of Subcommittee C and action of the Association, see *This Journal*, **31**, 51 (1948).

7.5 gm of gelatine 105 gm of water	*7.5 gm of gelatine 100 gm of water*
176	188
200	213
225	241
250	268
288	307

In 1924 the Edible Gelatin Research Society of America, Inc., an association of approximately 75 per cent of the American manufacturers of food gelatines, adopted the Bloom Gelometer and the Viscosity Pipette, and general procedures for the determinaton of jelly strength and viscosity as had been previously adopted by the National Association of Glue Manufacturers at their meeting at Atlantic City, October 10, 1923. The apparatus and methods of procedure as adopted were reported in *J. Ind. Eng. Chem.*, 16, 310–315 (1924). Later publications on these methods have appeared in the Analytical Edition *Ind. Eng. Chem.*, 2, pages 348–351 (1930). The Edible Gelatin Research Society of America, Inc. followed the identical procedure as outlined for glue, but instead of using 15 gm of glue for each 105 gm of water the Gelatin Association employs 7.5 gm of gelatin for each 105 gm of water.

This procedure and this concentration is now used by all American gelatine manufacturers and values recorded by these methods are accepted as general practice by nearly all American consumers. The methods may be considered as international because the British Association of Gelatin Manufacturers evaluate their products with the Bloom Gelometer at 7.5 gm for each 105 gm of water. Hence, in order that the A.O.A.C. methods may be in accord with national and international procedures for the determination of jelly strength, it is recommended* that sec. 9.6, page 104, 6th Ed. of the A.O.A.C. *Methods of Analysis*, be deleted and substitute adopted to read as follows:

"To 7.5 g of sample add from pipet, with stirring, 105 ml of water at 10° to 15°C. Let stand for one hour and then bring to 62° in 15 min. by placing in a water bath regulated at 65°; the sample may be swirled several times to aid solution. Finally mix by inversion, let stand 15 min., and place in a water bath controlled at 10° ±0.1°. Chill for 17 hours. Determine jelly strength on Bloom Gelometer (2)† adjusted for a 4 mm depression and to deliver 200 g shot/5 seconds (±5 g), using the ½" plummet."

REPORT ON MEAT AND MEAT PRODUCTS

By R. M. MEHURIN (Meat Inspection Division, Bureau of Animal Industry, Department of Agriculture, Washington 25, D. C.), *Referee*

For the reasons given by the Associate Referee on soybean flour and dried skim milk in meat products (ff.), it is apparent that it will prove very difficult to work out an accurate method for the determination of soybean flour. It is thought, therefore, that another Associate Referee should be appointed to take up the work on dried skim milk in meat products. The General Referee is already in possession of a satisfactory method for the determination of this substance, and there remains only the necessity of some minor experimental work before the method is ready for collaborative testing. It is hoped that an Associate Referee can be secured to conduct this work.

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* For report of Subcommittee C and action of the Association, see *This Journal*, 31, 51, 74 (1948).
† Reference number refers to the selected references, *Methods of Analysis*, 6th Ed., p. 104.

During the war, a method for the direct determination of creatin was developed which is reported to yield more accurate results than the official method, by which the creatin is determined by difference. It is believed this method should be studied during the coming year. The following recommendations* are made for work on Meat and Meat Products during 1948:

(1) That collaborative work be continued on the methods submitted by the Associate Referee for the determination of soybean flour in sausage and similar products.

(2) That an Associate Referee be appointed to continue work on a method for the determination of dried skim milk in sausage and similar products.

(3) That study be undertaken on a method for the direct determination of creatin.

No report on dried skim milk was given; see Referee report.

REPORT ON SOYBEAN FLOUR IN SAUSAGE AND SIMILAR PRODUCTS

By Oran L. Bennett (Meat Inspection Division, Bureau of Animal Industry, U. S. Department of Agriculture, Washington 25, D. C.), *Associate Referee*

The problem of developing a practicable method for the determination of soybean flour in meat food products is a complex one. This is in part due to the various added substances such as cereal, dried skim milk, spice, cures, etc., one or all of which may be added in the preparation of these products. Another difficulty in the way of development of an accurate method is the variation in the composition of commercial soybean flours, due to different methods of processing. This variation will obviously cause a difference in the results obtained by any method. This fact was demonstrated by Munsey[1] in his report on soybean flour in cereal products and confirmed by the author in his own work.

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* For report of Subcommittee C and action of the Association, see *This Journal*, 31, 52 (1948).

Several methods for the approximate determination of soybean flour in certain meat products have been published in the past several years but none of them satisfy the requirements for a practical analytical procedure for control work. This need has been accentuated by the recent decision of M. I. D. permitting sausage manufacturers to add soybean flour to their product.

The author, with the assistance of other interested chemists, has attempted to devise an approximate but rapid method which would be suitable for control work. In devising such a method, endeavors have been made to use the hemicellulose constituent of soybean flour as a means of its measurement, since knowledge gained through previous personal experience and consideration of published methods indicates this to be the best avenue of approach to the problem. Several methods, all of which utilized the hemicellulose component as means of measurement, were developed, but the two appearing to have the most merit were chosen for further study. The methods are not applicable to those products containing other materials of high cellulose content such as dried vegetables. Such ingredients are not permitted in Federally inspected sausage.

Each of the two methods employs a potassium hydroxide solution in alcohol to dissolve the meat from the soybean and cereal flour. Both methods are empirical and each employs a separate appropriate factor for conversion of percentage of hemicellulose found to percentage of soybean flour present.

Method I is an adaptation of Hendry's method[2] and was developed principally by chemists in the St. Louis Meat Inspection laboratory. This method is based on the fact that a certain proportion of the hemicellulose of soybean flour may be separated and converted by acid hydrolysis to nonfermentable reducing sugars, the amount of which, multiplied by a predetermined factor, indicates the amount of soybean flour present. Since lactose may be present through the inclusion of dried skim milk in the product, its removal is accomplished by extraction with hot water before digestion with the potassium hydroxide-alcohol solution. The hemicellulose contained in any cereal flour which may be present in sausage is also hydrolyzed to non-fermentable reducing sugars, but a correction is made for this, depending upon the amount of cereal present. While this is not a short method, it has been demonstrated that it yields fairly consistent results in the hands of different analysts.

Method II, developed by the author, also utilizes the hemicellulose constituent as a basis for measurement of soybean flour content. It takes advantage of the fact that the hemicellulose of soybean flour and the starch of the cereal flour may be separated by means of their different solubilities in different concentrations of hydrochloric acid. After separation the hemicellulose may be re-precipitated by the addition of 95% alcohol, centrifuged at a given speed for a period of time, and measured volumetrically. Volume in ml × a predetermined factor gives approximate % of soybean flour. The same factor is not applicable to product containing over 4 per cent of soybean flour. In such cases a smaller sample must be taken and a corresponding change made in the calculation. This method is comparatively short but sometimes gives results which are somewhat inconsistent.

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[1] *This Journal*, **30**, 187 (1947).
[2] *Ind. Eng. Chem., Anal. Ed.*, **11**, 611–613 (1939).

METHOD I

Weigh 10 gm of the finely chopped meat into a 100 ml centrifuge tube and add gradually, with constant stirring, 60 ml of hot water. Suspend the tube in a water bath, kept just below the boiling point, for 15–20 min. with occasional stirring. Wash off the rod with 2–3 ml of hot water. Centrifuge the tube 5 min., return to bath and add a slurry of 1 gm filtercel in 5 ml hot water. Stir thoroly without disturbing the meat and again centrifuge 10–15 min. Pour off and discard the supernatant liquid, which will probably be cloudy, especially in the presence of dried skim milk. Add 50 ml of 8% KOH in 95% alcohol to the tube, return to the water bath stirring until the alcohol boils. Reduce the temperature of the bath to ca 80 degrees C. and continue heating with occasional stirring for 10 min. after the particles of meat have disappeared (about 20 min. in the water bath is necessary). Filter the alkaline soln thru a Gooch crucible having a thin pad of asbestos covered with filtercel, using gentle suction and keeping the mixture well stirred during filtration. Wash the tube and crucible with four 25 ml portions of 95% alcohol. Remove the excess alcohol with strong suction. Invert the Gooch over a large stem funnel in a 200 ml Erlenmeyer flask and tap gently until the cake falls out. (The cake usually comes out in one piece although sometimes it will be necessary to remove the asbestos with a glass rod.) After moistening with a few ml of 2.5% HCl, break up the cake and wash it into the flask with a stream of 2.5% HCl from a wash bottle. Wash the centrifuge tube, Gooch, and rod, with the HCl using a rubber-tipped rod, and add the wash soln to the Erlenmeyer flask. The total volume of the acid soln used should approximate 60 ml. Thru a rubber stopper attach an air-reflex condenser to the flask. Suspend in a boiling satd salt soln for three hours, boiling temperature about 105°C. Remove from the salt bath, cool, and add 1 ml of 20% phosphotungstic acid. Neutralize to *p*H 6.5–7.0, first adding 6 ml of 25% NaOH, then complete neutralization with 10% NaOH from a dropper. Make to volume in a 100 ml flask and filter about 75 ml. Centrifuge, in a 100 ml tube, 10 ml of a 25% yeast suspension (Fleischmann or similar preparation). Discard the liquid and dry the sides of the tube. Add about 75 ml of the neutralized filtrate, shake, and allow to stand 1 hour with occasional mixing. Centrifuge until clear. Remove a 50 ml aliquot and determine reducing sugars according to the Munson-Walker method[3], using sodium thiosulfate for the titration. Subtract a blank of 2.3 mg of Cu_2O before computing dextrose. If no cereal is present in the sausage the per cent non-fermentable sugars as dextrose X 14.4 = per cent soybean flour in the sample. If cereal is present, 0.33% for each per cent of cereal must be subtracted from this total.

METHOD II

Weigh directly into a 100-ml oil tube (A.S.T.M. conical form, stem graduated from 0 to 3 in 0.1 ml), 10 g of finely divided sample. Add 50 ml of 8% soln of KOH

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[3] *Official and Tentative Methods of Analysis*, 6th Ed., (1945), p. 571, par. 34.38.

TABLE 1.—*Soybean flour in sausage*

ANALYST	METHOD NO. 1				METHOD NO. 2		
	SAMPLE NO.	SOYA FLOUR ADDED	CEREAL ADDED	SOYA FLOUR FOUND	SOYA FLOUR ADDED	CEREAL ADDED	SOYA FLOUR FOUND
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	1	1.0	0	.8	3.0	1.0	3.0
	2	2.0	0	2.1	1.0	1.0	1.2
	3	3.0	0	3.1	2.0	2.0	2.1
	4	4.0	0	4.3	3.0	3.0	3.2
	5	5.0	0	5.3	3.0	3.0	4.0
	6	3.0	1	3.3	4.0	4.0	5.1
	7	1.0	1	1.0	4.0	4.0	4.6
	8	2.0	2	1.8	4.0	4.0	5.1
	9	3.0	3	2.6	4.0	4.0	5.2
	10	4.0	4	3.8	5.0	5.0	4.8
2	1	3.7	2.7	3.6	3.7	2.7	3.8
	2	2.0	2.0		2.0	2.0	2.2
	3	2.9	2.4	2.7	2.9	2.4	
	4	3.6	3.2	3.1	3.6	3.2	4.0
	5	5.0	3.2	4.6	5.0	3.2	6.3
3	1	2.0	2.0	2.1	1.0	1.0	1.0
	2	2.0	2.0	2.3	3.0	2.0	3.3
	3	2.0	2.0	2.4	2.0	3.0	2.1
	4	2.0	2.0	2.0	1.0	1.0	.7
	5	2.0	0	2.0	2.0	2.0	1.6
	6	4.0	0	4.0	2.0	3.0	2.3
	7	3.0	0	2.5	1.0	1.0	.9
	8	4.0	0	4.1	0	2.0	0
	9	2.0	0	2.1	2.0	3.0	1.6
	10	2.0	0	1.9	3.0	2.0	2.7
4	1	2.5	1.0	2.8	1.0	1.0	.9
	2	2.5	1.0	2.6	1.0	2.0	.8
	3	4.0	2.0	3.8	2.0	3.5	1.8
	4	4.0	2.0	3.8	2.0	1.5	1.9
	5	4.0	1.0	4.2	3.0	2.5	3.0
	6	2.0	.0	2.1	3.0	0	3.1
	7	3.0	.0	2.9	3.0	0	3.3
	8	4.0	.0	4.0	3.5	2.0	3.4
	9	5.0	.0	5.0	3.5	0	3.6
	10	6.0	.0	6.1	4.0	1.0	4.3
5	1	3.0	0.0	2.6	0	0	0
	2	3.0	0.0	2.4	3.0	0	3.3
	3	3.0	3.0	2.1	3.0	0	3.3
	4	3.0	3.0	1.9	0	3.0	.6*
					3.0	3.0	3.0
					3.0	3.0	2.7
6	1	3.0	0	Dry 3.2	3.0	0	4.3
	2	3.0	0	Yeast 2.2	3.0	0	3.9
	3	3.0	0	Fresh 2.7	3.0	0	3.9
	4	3.0	0	Yeast 2.9	3.0	0	3.6

* This precipitate apparently was due to a small amount of hemicellulose from cereal present in sample.

in alcohol and digest on steam bath for 20 min., with occasional stirring to facilitate digestion. Remove from bath and make up to 100-ml mark with 95% alcohol. Shake well and centrifuge for 4 min. Decant and discard KOH-alcohol soln, and wash residue with 25 ml of 95% alcohol, using a glass rod to stir up the sediment. Centrifuge, decant, and discard alcoholic soln.

To the residue in tube add 50 ml of 1+3 HCl, and stir residue in stem with rod to reduce any lumpy material. Shake for one min., after which centrifuge for 4 min. at a speed of ca 2000 r.p.m. Add 25 ml of this clear soln to 75 ml of 95% alcohol in another oil tube (conical form, stem graduated from 0 to 1 in .05 ml), shake well, and let stand for 1 hour. Centrifuge for exactly 2 min. at a speed of 1500 r.p.m., accelerating to 1500 r.p.m. in 1 min. and using a head 6⅞ inches in diameter. (International Equipment Co., Catalog No. 235.) Read volume of residue in tube and multiply by the factor 6, to obtain the percentage of soybean flour present.

Results of analysis of samples of sausage containing known amounts of soybean flour by several analysts, using the two methods, are given in Table 1.

Samples of sausage containing various amounts of soybean flour were prepared by the Associate Referee and submitted to three collaborators for analysis. Results of these determinations are given in Table 2.

TABLE 2.—*Collaborative results*

	METHOD NO. 1—COLLABORATOR			METHOD NO. 2—COLLABORATOR		
	NO. 1*	NO. 2	NO. 3	NO. 1	NO. 2	NO. 3
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
Sausage containing 1% soybean flour		.6	1.3	.6	.7	1.1
Sausage containing 3% soybean flour		3.1	3.1	2.8	2.8	2.3
Sausage containing 1.5% soybean flour plus 1.5% cereal		1.1	1.9	.9	.9	1.6
Sausage containing 2% soybean flour plus 1.5% dried skim milk		1.9	2.1	1.7	1.6	2.0

* No analysis made.

While the two methods under study appear to yield only approximate results in their present form, it is believed that they are the best methods available for control work at this time. Further, your Associate Referee believes that these methods can be definitely improved by the adoption of some changes suggested by the collaborators and by further study.

It is recommended,* therefore, that work be continued along the same lines during the coming year.

ACKNOWLEDGMENT

Acknowledgment is made for valuable assistance in this work to collaborators H. R. Kraybill, American Meat Institute; M. L. Laing, Armour and Company; C. C. Zigler, Swift and Company; and to the several BAI meat inspection chemists who participated in this study.

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* For report of Subcommittee C and action of the Association, see *This Journal*, 31, 52 (1948).

REPORT ON MICROBIOLOGICAL METHODS

By GLENN G. SLOCUM (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

This is the first report of your Referee on Microbiological Methods since his appointment in May 1946. The past year and a half has been largely a period of orientation, and there is little to report in the way of progress. Much of the work on microbiological methods was necessarily curtailed or interrupted during the War and subsequently by the change in General Referee. During the next year work on these methods will be activated again and it is hoped that considerable progress can be reported at the next meeting.

The Sixth Edition of "*Methods of Analysis*" contains the following methods which have been adopted as official, first action:

1. Examination of Eggs and Egg Products.
2. Detecting and Estimating Numbers of Thermophilic Bacteria in Sugar.
3. Examination of Canned Vegetables.

The method for eggs and egg products is generally satisfactory but will require some slight modification and additional collaborative work. The method for sugar also appears to be satisfactory and probably can be made official in the near future. The same is probably true of the method for canned vegetables. However, methods for other non-acid canned foods, specifically canned meats and canned fish, have not been presented in form for proposal as tentative methods. It is highly desirable that methods for these products be developed along with the methods for canned vegetables and that they be so coordinated that the control laboratory will not be confronted with widely divergent procedures requiring a considerable variety of culture mediums and incubation times and temperatures. The latter is, of course, true for all microbiological methods.

The Sixth Edition of *Methods of Analysis* contains tentative methods for the examination of canned fruits and other acid canned food, and for nuts and nut products. The former method will need additional checking and possibly some modification and the appointment of an Associate Referee to fill immediately the vacancy created by the death of Mr. Linden is recommended. The methods for nuts and nut products have been under study by the Associate Referee and these studies will be continued during the year.

The report of the Associate Referee on frozen fruits and vegetables is being presented at this meeting. The General Referee concurs in this report.

In his reports as General Referee on Microbiological Methods, Dr. Hunter more than once referred to relationship of the American Public Health Association and this Association in the development of such methods. It is apparent that these organizations are following parallel courses; and it is imperative that a close coordination of effort must be obtained not only to avoid any conflict in procedures but also to prevent unnecessary duplication of effort by a proper division of labor. Dr. Hunter carefully laid the ground work for such coordination and your General Referee will continue to work to that end.

RECOMMENDATIONS*

It is recommended—

(1) That the methods in the report of the Associate Referee on frozen fruits and vegetables be studied further.

(2) That work be continued on eggs and egg products, sugar, canned vegetables, canned meat, canned fish, acid canned foods, nuts and nut products, and frozen fruits and vegetables.

No reports were given on microbiological methods in canned fishery products, canned meats, canned tomatoes and other acid vegetables and fruit products, or eggs and egg products.

REPORT ON MICROBIOLOGICAL METHODS FOR FROZEN FRUITS AND VEGETABLES

By H. E. Goresline (Production and Marketing Administration, Poultry Branch, Washington, D. C.), *Associate Referee*

The following methods for the examination of frozen fruits and vegetables are presented in order to form the basis for the development of complete methods. It is suggested that the few methods forming this report be printed, and that reprints be forwarded to at least 50 laboratories, colleges, experiment stations, and State and Government agencies, for the purpose of obtaining criticism and collaboration. At the end of a suitable trial period these groups will be contacted and the results of the findings reported to the Association in the form of recommended methods.

SUGGESTED METHODS FOR THE MICROBIOLOGICAL EXAMINATION OF FROZEN FRUITS AND VEGETABLES

EXAMINATION OF FROZEN VEGETABLES

From the lot to be examined select a suitable number of packages and transport in dry ice to the laboratory for analysis. Place the samples in a refrigerated (0°F.) storage chest until they are to be analyzed.

PREPARATION OF SAMPLE

A. Peas, lima beans, cut corn, whole or regular cut green beans, etc.

1. *Macroscopic examination.*—Open the package and note condition of the prod-

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* For report of Subcommittee C and action of the Association, see *This Journal*, 31, 53 (1948).

uct. If ice crystals are present on the inner walls of the package and the vegetables appear somewhat shriveled, it is indicative of thawing and subsequent refreezing, Record observations and note any abnormalities, such as unnatural color or odor. or pink colonies of torulae which are indicative of improper handling practices.

2. *Sampling for plate counts.*—Break the sample, if not loose-frozen, into small units by tapping the unopened package sharply against a table edge or by sharply striking the packing with a dull instrument, being careful not to break open the package. Open the package and remove sample with sterile spoon, taking the sample from various parts of the broken-out package, *e.g.*, from the center and corners.

Weigh a 50-g sample aseptically into a sterile borosilicate glass mechanical blender jar. Add 450 ml of sterile water to the jar and blend the contents of the jar for 2 mins. If a Variac transformer is available it is advisable to increase the speed of the motor gradually and then run the blender for the required time at the 100 V. setting of the transformer.

Allow the sample to stand for 2–3 min. to permit foam to subside.

Pipet 1 ml of the mixture into a 99 ml sterile water blank. Replace the cap on the diln bottle and shake the bottle briskly 25 times thru a 1-foot arc.

Pipet 1 ml aliquots of mixture into each of 2 petri dishes (1:1000 diln) and also 0.1 ml aliquots into each of 2 petri dishes (1:10,000 diln). (A 1:100 diln may be obtained by pipeting 0.1 ml aliquots of the original mixture into each of two petri dishes. Dilns. of 1:100, 1:1,000, and 1:10,000 will usually suffice for commercially packed frozen vegetables, although further dilns should be made if the history or the appearance of the samples warrants.)

Pour melted tryptone glucose extract agar (*p*H 7.0) cooled to 45°C. into the petri dishes immediately, and thoroly mix the diln water with the agar by gently rotating the plates in a figure 8 motion with slight tilting; harden, invert, and incubate at 32°C. for 4 days.

After the incubation period is complete count the plates, using a suitable colony counter. Record results as "Plate count of microorganisms per gram."

3. *Direct microscopic count method.*—The direct microscopic method has an advantage over the plate count method in that it is quicker and requires less equipment and glassware. Also it detects dead microorganisms, and indicates sanitary history, irrespective of the viable count.

Weigh 50 g of the vegetable into a 250 ml iodine flask.

Add 100 ml water, stopper flask, and shake briskly 50 times thru a 1-foot arc. Using a Breed pipet, transfer 0.01 ml of the washings to a microscope slide and using a needle spread the drop evenly over a one square centimeter area of the slide. Dry and fix with heat or with methyl alcohol. Stain with Gray's double dye stain (1), or with North aniline oil methylene blue stain (2), rinse, dry and examine under the microscope, using oil immersion. Use an ocular micrometer, such as a Wipple disc or Howard disc, with the microscope tube so adjusted that the side of the graduations is equal to 0.1 mm (area of field 0.01 sq mm). Count the cells in 100 fields, and multiply the number by 20,000 to bring to the gram sample basis. Express results as "Direct microscopic estimate, microorganisms per gram."

In the direct microscopic method the following assumptions are made. (A) That all the cells are removed from the surface of the vegetable by the washing. (B) that the suspension of bacterial cells is uniform, and (C) that the drop of liquid is evenly spread over one square centimeter.

B. Frozen Spinach

For proper comminution of spinach in a "blender" it is necessary to allow the package of spinach to defrost partially by standing at room temp. for 1½–2 hours. Open the package and weigh 50 g of the contents into a sterile borosilicate mechanical blender jar. Assemble the sample from various portions of the package taking care to select petiole and blade portions in about the same ratio as in the sample as a whole. Add 450 ml sterile water, blend for two min., and proceed as in analysis of A (peas, lima beans, etc.).

C. Frozen Broccoli and Cauliflower

Allow to defrost partially at room temp. Using a sterile scalpel cut portions from the curd and stem of several representative pieces of the vegetable. Aseptically transfer 50 g of these portions into the sterile borosilicate glass mechanical blender jars, add 450 ml sterile water, and proceed as directed for A (frozen peas, etc.).

D. Frozen Asparagus

Partially defrost at room temp. Cut spears into short lengths using sterilized scalpel. Transfer aseptically 50 g of the segments into a sterile borosilicate glass mechanical blender jar, add 450 ml sterile water and proceed as in analysis of A (frozen peas, etc.). When weighing out sample portions, a number of spears should be selected, and the proportion of butts and tips maintained.

(The "cuts and tips" type of asparagus pack can be weighed directly into the blender cup.)

EXAMINATION OF FROZEN FRUITS

PREPARATION OF SAMPLE

Hold the package of frozen fruit at room temp for 1–2 hours before opening. While the fruit is still partially frozen, cut portions from various parts of the contents of the package using a sterilized scalpel. The proportion of fruit to syrup should approximate that of the whole package. Weigh 50 g of fruit and syrup into a sterile borosilicate glass mechanical blender jar, add 450 ml sterile water and blend for two min. Make a 1:1000 diln by adding 1 ml of the blended mixture to 99 ml sterile water and further dilns in the usual manner. Plate 1 ml portions from the various dilns on tryptone glucose extract agar, and incubate for 3 days at 32°C. Count colonies under a suitable colony counter, and record results as "Plate count of microorganisms per gram."

Make direct microscopic counts for molds and yeasts according to the methods given in Official Methods, A.O.A.C. (3) for microscopic analysis of tomato products. Care must be taken to distinguish between mold hyphae and fruit setae.

REFERENCES

(1) Gray, P. H. H., *J. Milk Tech.* **6** (2); 76 (1943) "Two-Stain Method for Direct Bacteria Count."

(2) North, W. R., *This Journal*, **28**, 424 (1945), "Aniline Oil—Methylene Blue Stain for the Direct Microscopic Count of Bacteria in Dried Milk and Dried Eggs."

(3) *Methods of Analysis, A.O.A.C.*, 6th Ed., 1945, p. 788.

REPORT ON NUTS AND NUT PRODUCTS

By A. M. Henry (Food and Drug Administration, Federal Security Agency, 416 Federal Annex, Atlanta 3, Ga.), *Referee*

The work on methods for nuts has been of an exploratory nature. There is no satisfactory method of preparing very oily nut meats for analysis. All methods in the chapter on nuts are tentative. As there appears to be greater need of methods for moisture, or volatile matter, and fat these were selected for study.

INSTRUCTIONS TO COLLABORATORS

Preparation of Sample

Preserve sample in glass-top fruit jar or similar air-tight container at 35 to 50°F. or 5 to 10°C. Prepare sample for analysis as follows:

(*A-1*) *Nuts in Shell.*—Remove meats from shells, being careful to remove all particles of shells from the meats, and prepare meats as in A-2.

(*A-2*) *Nut Meats, Shredded Coconut, and Similar Sized Pieces.*—Grind not less than 250 grams twice thru Enterprise No. 5 food chopper, equipped with a revolving knife blade and plate with holes ca ⅛ inch in diameter. Any other make of food chopper that will give similar results may be used. The nut meats may also be comminuted by a mechanical grating device instead of food chopper that will comminute to similar condition and size of pieces. Mix ground sample well and store in air-tight glass container. (Comments on any other equipment that will satisfactorily prepare nut meats will be appreciated.)

(*B*) *Nut Butters and Pastes.*—Transfer sample to container of convenient size and mix carefully with stiff bladed spatula or knife. A semi-solid product may be warmed and then mixed with an electric malted milk mixer or similar equipment. Store sample in air-tight glass container.

Moisture Determinations

Method 1: Determine moisture by A.O.A.C. Method **27.3**. If necessary to secure a thin layer of the material, add a few ml of H_2O or ethanol and mix thoroughly.

Method 2: Same as Method 1 except dry at 70 to 75°C.

Method 3: Determine moisture by A.O.A.C. Method **27.4** *and* **27.5**, except substitute 500 ml Florence flask for 250 ml Erlenmeyer flask, as it is necessary to use 100 gm sample for most nut products.

Fat Determinations

Method 1: Determine fat by A.O.A.C. **27.24** and **27.25**, using Soxhlet extractor. Use the dried residue from moisture determination, Method 1 or Method 2.

Method 2: Determine fat as directed under A.O.A.C. **19.23**, using 250 ml extraction flask instead of 150 ml Erlenmeyer flask. On nut butters or pastes it may be desirable to use equal volume of filter-cel to facilitate filtration. No work is desired on fat constants or proteins at this time. In place of the Knorr extraction tube, a fritted glass Büchner funnel may be used, but in such case it is desirable to use a layer of the purified asbestos next to the fritted glass. A 30 ml Büchner type funnel with fritted disc of coarse or medium porosity, listed in Corning Catalogue as 38060, or an Allihn tube with fritted disc of medium porosity listed in Corning Catalogue as 37730, is suitable.

Method 3: Determine by A.O.A.C. Method **30.6**. Do not use any of this method beyond the sentence in line 6: "Dry dish and contents at 100° for 30 minutes, cool, and weigh."

The results are given in Tables 1 and 2.

COLLABORATIVE RESULTS

T. A. Pickett reported results on Methods 1 and 2 for drying five hours instead of drying to constant weight. He also reported on drying for five hours at the temperature of boiling water, with results 7 to 10 per cent lower on peanuts and 33 per cent lower on peanut butter; and for drying at 135°C. for two hours, with results 2 to 3 per cent higher for peanuts and 10 per cent higher for peanut butter.

TABLE 1.—*Collaborative results on methods for moisture in nuts*

PRODUCT AND COLLABORATOR	METHOD 1		METHOD 2		METHOD 3	
	per cent		*per cent*		*per cent*	
Almonds:						
Munday	4.78	4.81	4.36	4.35	4.6	4.6
Coconut:						
Munday	6.39	6.29	5.96	5.85	6.5	6.4
Peanuts:						
Pickett, Sample #1	6.86	6.86	6.35	6.47	6.36	—
	6.93	6.88	6.35	6.45		
Sample #2	6.70	6.60	6.01	6.19	—	—
	6.66	6.72	6.01	6.11		
Sample #3	6.10	6.03	5.51	5.62	—	—
	6.06	6.06	5.60	5.53		
Pecans:						
Rokita	2.91	2.93	2.73	2.75	3.0	3.0
Walnuts:						
Banes	4.36	4.35	3.89	3.79	4.42	4.28
Peanut Butter:						
Banes	1.73	1.76	1.27	1.30	1.81	—
Munday	1.46	1.45	1.03	1.07	1.9	1.9
Pickett	1.47	1.60	1.12	1.10	1.67	1.62
	1.43	1.53	1.05	1.06		
Rokita	1.28	1.30	1.04	1.00	1.8	—

The "Rapid Method for Determining Oil Content of Tung Kernels" by Joseph Hamilton and Seymour G. Gilbert[1] suggests the use of a fat solvent in a Waring Blendor as an aid in determining fat or oil in nuts. Ella M. Baer and Sylvia F. Hoisington report results by the above method as well as by a modification of Method 1 wherein they use a Waring Blendor with petroleum ether for disintegrating the sample before extraction in the Soxhlet apparatus. They obtain higher results with the

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[1] Analytical Chemistry, Vol. 19, p. 453.

Tung kernel method but report trouble with colloidal material that does not settle. Mrs. Rokita reports that method **30.6** is not suitable to nut butters, as it is hard to transfer the sample to the flask without loss of chloroform by volatilization, which tends to give high results. Method **19.28** gives low results, probably from incomplete extraction due to large particles. Mrs. Rokita and others report trouble with Method **27.25** on peanut butter, as starch contaminates the extract and it is necessary to treat the extract with petroleum ether.

TABLE 2.—*Collaborative results on methods for fat in nuts*

PRODUCT AND COLLABORATOR	METHOD 1		METHOD 2		METHOD 3	
	per cent		*per cent*		*per cent*	
Almonds:						
Munday	55.24	55.00	54.41	54.18	51.87	52.18
Coconut:						
Munday	45.42	45.76	33.02	33.97	33.72	33.89
Peanuts:						
Pickett, Sample #1	45.94	46.14	45.40	44.92	45.80	46.40
	46.01		45.16		46.90	
Sample #2	45.37	45.47	43.90	43.87	45.62	45.97
	45.38		43.38		46.05	
Sample #3	47.71	47.62	45.48	46.29	48.03	48.92
	47.62	47.70	44.29		48.77	
Pecans:						
Rokita	73.41	73.81	70.38	70.30	73.2	73.3
Walnuts:						
Banes	71.0		57.7		63.0	62.9
Peanut Butter:						
Banes	51.0		48.0		49.8	49.6
Munday	56.77	56.53	49.91	50.11	54 01	63.71
Pickett	47.31	47.52	47.28	47.59		
	48.25	47.92	47.94	47.12		
Rokita	50.47	50.09	48.34	48.49	52.2	53.7

Drying at 70°C. in vacuum gives lower results than at 100°C. in vacuum or distillation with toluene. Mr. Munday and Mrs. Rokita report that under some conditions the toluene distillation method gives high results which may be due to high humidity.

In the preparation of the sample, Baer and Hoisington recommend the Waring Blendor. The Griscer grater has been recommended for preparing cheese samples and is probably suitable for nuts. Hamilton and Gilbert recommend a flaking machine. Small-sized food choppers have also been used for preparing nut samples for analysis.

Grateful appreciation is expressed to the following collaborators who participated in this work:

Ella M. Baer, Florida Agricultural Experiment Station, Gainesville, Fla.
Daniel Banes, U. S. Food and Drug Administration, Chicago, Ill.
Sylvia T. Hoisington, Florida Agric. Experiment Station, Gainesville, Fla.
William H. Munday, U. S. Food and Drug Administration, Kansas City, Mo.
T. A. Pickett, Georgia Experiment Station, Experiment, Ga.
Phyllis B. Rokita, U. S. Food and Drug Administration, Atlanta, Ga.

RECOMMENDATIONS*

It is recommended—

(1) That methods for preparation of sample be studied.
(2) That methods for moisture or volatile matter be studied.
(3) That methods for fat determination be studied.
(4) That the other methods be studied if time is available.

REPORT ON VEGETABLE DRUGS AND THEIR DERIVATIVES

By PAUL S. JORGENSEN (Food and Drug Administration, Federal Security Agency, San Francisco 2, Calif.), *Referee*

Only three Associate Referees sent reports, as the following will indicate.

RECOMMENDATIONS†

Chemical Methods for Ergot Alkaloids.—No report was received. It is recommended that the subject be continued.

Physostigmine in Ointments.—A report was received describing an improved procedure for the determination of physostigmine in tablets with the recommendation that the improvement be incorporated into the present tentative method and that it then be adopted as official (first action). It was further recommended that work on the determination of physostigmine in ointments be continued. The Referee concurs in these recommendations.

Theobromine and Phenobarbital.—A progress report was received by the Referee. No final report was received. It is recommended that the subject be continued.

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* For report of Subcommittee C and action of the Association, see *This Journal*, **31**, 53 (1948).
† For report of Subcommittee B and action of the Association, see *This Journal*, **31**, 45 (1948).

Aminopyrine, Ephedrine, and Phenobarbital.—No report was received. It is recommended that the subject be continued.

Quinine.—No report was received, but the Associate Referee in correspondence outlined progress in the comparison of the method described by Herd[1] with the A.O.A.C. method (pages 669 and 670). This report also included a statement regarding a new quantitative procedure which may prove to be better than either of the two methods referred to above. The Referee recommends that the subject be continued.

Ephedrine.—A report was received. It is recommended that the subject be continued.

Spirit of Camphor.—A report was submitted. The Associate Referee recommends that the proposed modification of the N.F. VIII method be adopted as tentative and that the subject be closed. The Referee concurs.

Chemical Methods for Penicillin.—No report was received. It is recommended that the subject be continued.

REPORT ON PHYSOSTIGMINE SALICYLATE AND PHYSOSTIGMINE IN OINTMENTS

By MATTHEW L. DOW (Food and Drug Administration, Federal Security Agency, St Louis, Mo.), *Associate Referee*

The present official method for the determination of physostigmine salicylate is dependent upon an accurate titration of the free base which has initially been completely extracted from sodium bicarbonate solution with chloroform. G. M. Johnson (1), the author of the method, and his collaborators were able to obtain consistent, but slightly low (97.5%) recoveries (2) of physostigmine from a lactose mix, taking the precaution only to avoid carbonate error in titration and to completely extract the alkaloid from sodium bicarbonate solution. The estimation of the methyl red end point was left to the judgment of the analyst.

Kolthoff (3) reports the stoichiometric point of physostigmine to occur at a *p*H of 5.0. Johnson checked this figure electrometrically using pure physostigmine and arrived at a value of 4.8, or just on the acid side of the methyl red end point. The shape of his titration curve near the equivalence point indicated that either another indicator should be used or that the color of the methyl red indicator should be checked against that of a buffered solution. The latter proved to give the best results and was therefore incorporated into the method.

A dry mixture of lactose and 2.43 per cent by weight of physostigmine salicylate was prepared and submitted for collaborative study. The purity of the alkaloid based on the nitrogen content was 99.5 per cent.

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[1] *J. A. Ph. A., Scien. Ed.,* XXXI, No. 1, Jan. 1942.

DETERMINATION

Follow *Methods of Analysis* 39.99 thru the sentence "Add methyl red indicator 39.8(b), and titrate the excess acid with 0.02 *N* NaOH." Then insert the following "until the color of the soln exactly matches that of 15 ml of standard buffer (*p*H =5.0; 50 ml M/5 KH Phthalate plus 23.65 ml. M/5 NaOH diluted to 200 ml) containing one drop of methyl red indicator or until a *p*H of 5.0 is reached as indicated by a *p*H meter." Finally continue with last sentence, "1 ml of 0.02 *N* H_2SO_4, etc."

TABLE 1.—*Collaborative results*

COLLABORATOR	PHYSOSTIGMINE SALICYLATE FOUND	RECOVERY
	per cent	*per cent*
J. H. McGee,	2.36	97.1
St. Louis, Mo.	2.36	97.1
	2.33	95.9
S. D. Fine,	2.39	98.3
Cincinnati, Ohio	2.32	95.5
H. R. Bond,	2.43	100.0
Kansas City, Mo.	2.42	99.6
	2.40	98.8
W. F. Kunke,	2.31	95.1
Chicago, Ill.	2.35	96.7
	2.36	97.1
G. S. Keppel,	2.42	99.6
Minneapolis, Minn.	2.45	100.8
M. L. Dow,	2.45	100.8
St. Louis, Mo.	2.39	98.3
	2.39	98.3
Ave.	2.37	97.9

The results indicate that slightly better recoveries are possible by using the revised method.

An experiment was conducted to determine whether physostigmine was hydrolyzed by the sodium bicarbonate to any extent. A portion of the lactose mix was allowed to remain in an excess of sodium bicarbonate solution for varying periods of time up to one hour without any untoward results.

In view of this and the consistency of the collaborators' results, it is recommended that the revised method used above be adopted as official (first action).

This proposed method was applied to the determination of physostigmine in ointments with very unsatisfactory low results. In addition the tentative prostigmine method (4) and a standard acid extraction method were tried upon the suggestion of the General Referee, but the results were too erratic to be of any use.

The ointment was prepared by mixing powdered physostigmine salicylate into a molten mixture of lanolin and petrolatum and stirring for several minutes after congealing had taken place. Three determinations using the above proposed method (the ointment was first dissolved in ether) were made and low results were obtained. The ointment was then spread out on a flat plate and mixed for ten minutes with a glass rod. The determinations were repeated with the same erratic low results. The ointment was stirred thoroughly for a third time and the determinations repeated with no better results.

It is apparent that some way must be found to obtain complete recovery of the alkaloid from the ointment. Ether, chloroform, and petroleum benzene were used to dissolve the ointment with about the same recoveries obtained in each instance. Ether has the advantage of producing less emulsions. An easily and accurately determined "tracer" compound mixed in with the ointment was contemplated but was not tried because of the lack of time.

It is recommended* that the work on the determination of physostigmine in ointments be continued, with particular attention being paid to obtaining complete recovery of the alkaloid from the ointment.

LITERATURE CITED

(1) George M. Johnson, *This Journal*, **14**, 816 (1941).
(2) ———, *Ibid.*, **14**, 817 (1941).
(3) J. M. Kolthoff, "Die Dissoziationskonstante, das Loslichkeits product und die Titnerbarkeit von Alkaloiden."
(4) *Methods of Analysis*, A.O.A.C., 6th Ed., p. 693.

REPORT ON EPHEDRINE

By Llewellyn H. Welsh (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

At the 1946 meeting of the Association, the Associate Referee proposed a general method[1] for the determination of ephedrine in inhalants, nasal jellies, syrups, and solutions. Results obtained on preparations of known composition and on products produced by various pharmaceutical companies indicated that the method was of value, and it was recommended that collaborative study be undertaken. The present report includes the results of collaborative study on three different samples, each corresponding to at least one dosage form of the drug.

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* For report of Subcommittee B and action of the Association, see *This Journal*, **31**, 45 (1948).

[1] *This Journal*, **30**, 467 (1947).

Sample I consisted of pure, recrystallized 3,4-dimethyl-2,5-diphenyloxazolidine, m.p. 72–73° (corr.), prepared by reacting benzaldehyde with *l*-ephedrine base.[2] On acid hydrolysis it yields ephedrine theoretically equivalent to 65.22 per cent of its weight. This substance is used in some inhalants containing a light liquid petrolatum base. Since observations in this laboratory have indicated that the compound undergoes some decomposition when liquid petrolatum solutions of it are stored, with the result that recovery figures for ephedrine are low, collaborators were instructed to mix the sample with liquid petrolatum when beginning the analysis. Results of collaborative work on this sample correspond to those obtainable from oily solutions of ephedrine which has been chemically combined with carbonyl compounds, and are also representative of those obtainable from solution of the free base in aromatized oils.

Sample II had the following composition: ephedrine sulfate 0.4400 g. Syrup of Cherry N.F. 90 ml, ethanol (95%) 22 ml, water *q.s.* 200 ml. This mixture contained the equivalent of 0.1697 g ephedrine base per 100 ml and approximately 12% of alcohol by volume. It contained the lowest concentration of ephedrine and the highest concentration of ethanol which the Associate Referee has encountered in commercial ephedrine syrups, and it was chosen for collaborative study because it seemed reasonable that if the method proved satisfactory for this mixture it probably would be suitable also for similar preparations containing less alcohol and more ephedrine.

Sample III was a mixture of ephedrine hydrochloride (10%) and potato starch (90%) and contained the equivalent of 8.192% of ephedrine base. This composition was prepared to simulate those of tablets and the contents of capsules. At this point, the use of sodium carbonate in the assay instructions for sample III might be explained. In the report of last year,[1] the statement was made that although the method had not as yet been applied to tablets and capsules of ephedrine salts, no difficulty was anticipated with these dosage forms. It was found, however, that when aqueous suspensions of potato starch were made alkaline with sodium hydroxide there resulted a semi-solid translucent gel which could not possibly be extracted successfully by shaking with solvents. The use of sodium carbonate instead of the hydroxide provided a *p*H high enough to liberate ephedrine base but not sufficiently high to cause swelling of the starch grains and consequent jellification.

INSTRUCTIONS TO COLLABORATORS

The directions followed by the Associate Referee and sent to collaborators 1, 2, and 4 were as follows.

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[2] *J. Chem. Soc.*, 1932, 1580.

Sample I. Accurately weigh a sample of about 0.15 gram and dissolve it in 5 ml of benzene contained in a 125 ml Erlenmeyer flask. After mixing the solution with 10 ml of light mineral oil, add 10 ml of 5% sulfuric acid and frequently agitate and swirl the mixture while it is boiled for ten minutes on a hot plate. Cool the flask, transfer the contents to a separatory funnel, and rinse the Erlenmeyer with portions of benzene totaling about 15 ml in order to remove all oily matter from it. It is desirable that the flask be provided with a lip in order to facilitate the quantitative transfer of the contents to the funnel. Shake the funnel containing the acid and benzene rinsings, drain off the acid layer into a second separatory funnel, and extract the benzene-oil phase with three 5 ml portions of water which have been previously used to rinse the flask. (In the transfer of ephedrine from organic solvent to aqueous phase, and *vice versa*, shakeouts should be conducted for a full minute.) Wash the aqueous soln of ephedrine sulfate with 3 ml of chloroform, add 2.5 ml of 20% sodium hydroxide, and extract the ephedrine with four 15 ml portions of chloroform. Filter the extracts thru a pledget of cotton into a tared 100 ml beaker, previously dried at 110° and cooled in a desiccator, rinse the cotton with chloroform and add five drops (0.2 ml) of conc. hydrochloric acid to the filtrate and washings. Heat the beaker on a steam bath in a current of air until the volume of liquid has been reduced to about 1 or 2 ml. Then cautiously heat, without air current, until the odor of hydrogen chloride has disappeared and the residue is apparently dry. Heat the beaker in an oven at 110° for one-half hour, cool in a desiccator and weigh: weight of residue $\times 0.8192 =$ weight of ephedrine base. Report results as per cent of ephedrine base in sample.

Sample II. Measure out 10 ml of sample in a 10 ml volumetric flask and transfer to a separatory funnel by means of small portions of water totaling 10 ml. Make the mixture alkaline with about 3 ml of 20% sodium hydroxide and quantitatively extract the ephedrine with five 20–25 ml portions of ether. Shake the combined ether extracts with 5 ml of 10% sulfuric acid, then with four 5 ml portions of water, and continue the assay as described for Sample I, beginning with the chloroform wash of the acid soln, up to the point at which the chloroform extracts are evaporated in the presence of hydrochloric acid in a 100 ml beaker, which, in this case, need not be tared. Continue evaporation until the chloroform, but not the excess hydrochloric acid, has been removed. Direct a fine stream of redistilled reagent grade methanol around the inside of the beaker to dissolve the hydrochloride, and repeat the process immediately with a stream of chloroform. Transfer the methanol-chloroform soln to a tared 20 ml beaker, previously dried at 110° and cooled in a desiccator, and repeat the methanol and chloroform rinsings until the ephedrine salt has been quantitatively transferred. Evaporate the soln on a steam bath, in a current of air, until the salt begins to crystallize. Continue the removal of solvent by cautious heating, to avoid loss from crepitation, until the residue is apparently dry and there is no odor of hydrogen chloride. Dry the residue as previously described and weigh. Report results as grams ephedrine base/100 ml.

Sample III. Accurately weigh a sample of about 1.0 gram, suspend in 20 ml of water in a separatory funnel, add 0.5 gram of anhydrous sodium carbonate, and, beginning with the ether extractions, proceed with the assay as described for Sample II with the exceptions that the acid extract of ephedrine sulfate is basified with 1.0 gram of anhydrous sodium carbonate, and that the chloroform extract plus hydrochloric acid is evaporated in a tared 100 ml beaker as described for Sample I. Report results as per cent ephedrine base in sample.

Using an Anschütz or calibrated thermometer, determine the capillary melting point of one residue from each sample.

As a result of consideration of the results and comments of these collaborators, the original directions were modified. The modification included a test for complete extraction of the drug, and instructed: that care be exercised to avoid superheating and bumping in the acid hydrolysis of the oxazolidine; that all shakeouts be conducted as vigorously as practicable; that, instead of adding a specified quantity of sodium hydroxide or carbonate to acid extracts of ephedrine sulfate prior to extraction of the base, the acid extracts be rendered slightly alkaline before adding a specified excess of alkali. The modified instructions were sent to collaborators 5 and 6.

COMMENTS OF COLLABORATORS

Charles F. Bruening.—"On Sample I . . . some determinations were lost because of bumping . . . . Possibly the method can be improved by using beads and constantly agitating to eliminate bumping."

Harry Rogavitz.—"Sample I. Boiling a two phase system for 10 minutes on a hot plate is difficult to control. Despite constant agitation, one determination was ruined because of superheating and loss of sample. Unless boiling is absolutely essential for success of the method, it is suggested that the directions be modified so that the sample would be warmed on steam bath for a period of time.

"Instead of the specific direction 'add 2.5 ml of 20% sodium hydroxide,' it is suggested that some general direction be used, as, 'make the soln alkaline to litmus with about 2.5 ml of 20% sodium hydroxide.' Because of the possible variation in strength of the reagents as ordinarily prepared in the laboratory, there is not enough margin to insure alkalinity when 2.5 ml of 20% sodium hydroxide are added to 10 ml of 5% sulfuric acid. In the actual determinations, it was found that after extraction with chloroform, one of the residual aqueous solns was faintly alkaline to litmus and the other was neutral. There was quite a discrepancy in the weights of the residues obtained. The residual aqueous solns were made distinctly alkaline to litmus by the subsequent addition of 0.5 ml of 20% sodium hydroxide and further chloroform extractions were made. When the weights of residues from supplemental extractions were added to that obtained from original extractions, concordant results were obtained. The data reported represent the sum of the original and supplemental extractions.

"If a general method should be adopted, it is suggested that some test for complete extraction be included, as 'Insure complete extraction by a fifth shaking with 15 ml of chloroform, addition of 0.2 ml conc. hydrochloric acid, and evaporation of the solvent in a separate container.' "

"Sample II. On heating residue in oven at 110°C., the residue turned brownish."

William F. Kunke.—"The results were obtained by following the respective method, particularly with reference to the volumes and the number of extractions, the length of time of each shaking was very appreciably more than one minute.

"In the case of Sample III it appears advisable to report the results found by making four more successive chloroform extractions of 15 ml each. The weights of the ephedrine hydrochloride were 0.0081 gram and 0.0028 gram, respectively. Including these results gives the total ephedrine base content as 7.94% and 7.85%.

"The incomplete extraction of the ephedrine base by four successive chloroform extractions of 15 ml each was confirmed by duplicate controls using 0.0958 gram ephedrine hydrochloride, 5 ml of 10% sulfuric acid, 20 ml of water, and 1.0 gram of anhydrous sodium carbonate."

Paul S. Jorgensen.—"Residues from each sample after drying at 110°C. for thirty minutes were slightly brown in color."

Sidney Gottlieb.—"It would seem advisable to routinely call for six extractions

instead of four in the final chloroform extraction. I found appreciable amounts of residue in the fifth and sixth extractions in all the samples, but negligible amounts in the seventh and eighth."

DISCUSSION

Five of the six collaborators, including one with no previous experience in proximate assays, obtained results on Sample I which ranged between 98.1 and 101.0% of the theoretical. These results are considered very good, and the average of 98.6% for all collaborators is considered satisfactory.

Results of six collaborators ranged between 96.1 and 108.4% for Sample II. Of the four collaborators who were sent the original instructions, the results of three were in the range of 100.8–103.1%, while those of the fourth ranged from 96.1–100.4%. The fact that the results of the remaining two collaborators, whose modified instructions included testing for complete extraction, obtained results of from 106.1 to 108.4% suggests that the apparently better results of the first group were due to the compensating factors of incomplete extraction of ephedrine and the presence of extraneous matter in the residue. As applied to syrups of ephedrine content as low as that of this collaborative sample, the method really represents an attempt to gravimetrically determine semimicro amounts of ephedrine by means of a proximate assay. The residues of ephedrine hydrochloride amounted to approximately 20 mg., and a difference of 1 mg. resulting from the presence of substances having solubilities similar to those of ephedrine, or arising for any other reason, would represent an error of 5 per cent. Increasing the size of the sample in order to obtain a much larger residue is an obvious recourse. Although this would render the method more cumbersome, it might represent an over-all advantage. From the collaborative results, it must be concluded that, when applied to syrups of composition similar to that of the collaborative sample, the method tends to give results some 5 per cent higher than those usually considered acceptable. However, in view of the fact that there are no official methods of analysis for ephedrine syrups of any type, the method might be adopted on a tentative basis.

Four of the six collaborators obtained what the Associate Referee considers to be good results (97.4–99.2%) on Sample III, and the average for the six collaborators was 97.5%. Two of the collaborators reported results representing 94.4–96.9% recoveries. The directions sent to Collaborator 4 did not include a test for complete extraction of the alkaloid and did not direct that a measured excess of carbonate be added before undertaking the chloroform extraction. Although his results include the ephedrine hydrochloride residue obtained from four additional extractions, it is not known whether complete extraction of ephedrine was effected, and it is possible that additional extraction would have raised the recovery figures. Modified instructions were sent to Collaborator 6,

TABLE 1.—*Results of collaborators*

COLLABORATOR	SAMPLE	EPHEDRINE BASE FOUND	PER CENT RECOVERY	M.P. OF RESIDUE °C. CORR.
		per cent		
1. Charles F. Bruening, U. S. Food & Drug Admin., Baltimore, Md.	I	64.8	99.4	217.2–218.0
	II*	0.173	101.9	215.4–216.8
		0.173	101.9	
	III	8.11	99.0	218.0–218.5
		8.13	99.2	
2. Harry Rogavitz, U. S. Food & Drug Admin., New York, N. Y.	I	65.9	101.0	216–217
		65.9	101.0	
	II*	0.174	102.5	213–216
		0.175	103.1	
	III	8.11	99.0	214–216
		7.98	97.4	
3. L. H. Welsh, U. S. Food & Drug Administration, Washington, D. C.	I	64.0	98.1	216–217.5
		64.2	98.4	
		64.5	98.9	
	II*	0.171	100.8	214–217
		0.172	101.4	
	III	8.03	98.0	216–217
		8.03	98.0	
4. Wm. F. Kunke, U. S. Food & Drug Administration, Chicago, Ill.	I	64.7	99.2	216–219
		64.6	99.0	
	II*	0.1704	100.4	
		0.1654	97.5	218–220
		0.1630	96.1	
	III	7.94	96.9	
		7.85	95.8	218–219
5. Sidney Gottlieb, U. S. Food & Drug Administration, Washington, D. C.	I	65.2	100.0	217.5–219
		65.7	100.8	
	II*	0.180	106.1	214.5–216.5
		0.181	106.7	
	III	8.07	98.5	217–218.5
		8.12	99.1	
6. Paul S. Jorgensen, U. S. Food & Drug Administration, San Francisco, Calif.	I	61.08	93.7	219.5†
		61.03	93.6	
	II*	0.184	108.4	216.5†
		0.182	107.2	
	III	7.73	94.4	219†
		7.75	94.6	

* For sample II, results shown as g/100 ml.
† Uncorrected, not included in m. p. average.

however, and it is assumed that the extraction test was used and that the transfer of ephedrine from water to chloroform was complete. Low recoveries would then most probably be due to failure to attain equilibrium in the ether extractions or be the result of incomplete separation of the aqueous and ethereal phases.

Average melting ranges of Sample I (216.5–218.5°) and Sample III (216.5–218.0°) fall within the 216–220° limits of the U.S.P. XII, although in one case the beginning of fusion of the residue from Sample III was reported as two degrees lower than the U.S.P. minimum. The lower purity of residues from Sample II is reflected in the average melting range (215.0–217.5°), in which the beginning of fusion is 1.5° lower than the averages for the other two samples. Also indicative of the lower purity of Sample II residues are the high recovery percentages reported and the fact that Collaborators 1, 2, 3, and 5 observed that residues from this sample were more highly colored than those from the other samples.

The instructions sent to the collaborators were reworded into a general method, in which modifications are described for adapting the method to dosage forms (water-soluble jellies, petroleum jellies, solutions of ephedrine salts, etc.) which were not subjected to collaborative study. Complete details of the method have been published in *This Journal*, **31**, 113 (1948). It has been adopted as official, first action. Samples representing these three types of preparations were not included in the study because it was felt that their inclusion would unduly burden the collaborators. The majority of the collaborative figures (Table 1) reasonably parallel those of the Associate Referee, who obtained satisfactory results in adapting the method to the three dosage forms in question. In the opinion of the Associate Referee, the adaptations are of a nature which would justify the conclusion that a collaborative study on these dosage forms would yield results paralleling those of the present study.

RECOMMENDATIONS*

It is recommended that the proposed method for the determination of ephedrine in its various dosage forms be adopted as tentative and that the subject be closed.

No reports were given on chemical methods for ergot alkaloids, on theobromine and phenobarbital, or on aminopyrine, ephedrine, and phenobarbital; for quinine, see report of Referee.

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* For report of Subcommittee B and action of the Association, see *This Journal*, **31**, 45 (1948).

REPORT ON SPIRIT OF CAMPHOR

By H. W. Conroy (Food and Drug Administration, Federal Security Agency, Kansas City, Mo.), *Associate Referee*

Spirit of Camphor was a U.S.P. XII product, but the preparation was omitted from the U.S.P. XIII. The same product and assay is now official in the National Formulary VIII.

Work this year was confined to a collaborative study of the determination of camphor by the official National Formulary VIII assay and by a modification of that assay. The latter method was sent to collaborators in a revised form from its original (*This Journal*, **28**, 719, 1945). Reagents specified were the same, except that following "(2) Aldehyde-free alcohol," the words "U.S.P. XII, p. 649," were deleted.

Details of the method are published in *This Journal*, **31**, 115 (1948).

COLLABORATIVE WORK

One sample of Spirit of Camphor, containing 9.60 g camphor in 100 ml at 20°C., was sent to collaborators. The camphor used in the prepara-

Table 1.—*Collaborative results*

COLLABORATOR*	CAMPHOR		RECOVERY	
	N.F. VIII METHOD		MODIFIED N.F. VIII METHOD	
	g/100 ml	*per cent*	*g/100 ml*	*per cent*
M. L. Dow	9.49	98.9	9.50	99.0
	9.43	98.2	9.53	99.3
			9.51	99.1
Sidney Williams	8.07	84.1	9.55	99.5
	8.42	87.7	9.56	99.6
	8.12	84.6	9.58	99.8
Rupert Hyatt	9.27	96.6	9.56	99.6
	9.08	94.6	9.60	100.0
Daniel Banes	8.34	86.9	9.52	99.2
	8.32	86.7	9.56	99.6
J. A. Thomas	9.13	95.1	9.49	98.9
	9.16	95.4	9.48	98.8
	8.99	93.6	9.49	98.9
Associate Referee	8.93	93.0	9.55	99.5
	9.05	94.3	9.58	99.8
Averages	8.84	92.1	9.54	99.4

* Addresses in order of listing: St. Louis, Mo., Minneapolis, Minn., Cincinnati, Ohio, Chicago, Ill., New Orleans, La., Kansas City, Mo. All of the U. S. Food and Drug Administration.

tion was purified according to C.A. **34,** 7302, 1940, and aldehyde free alcohol was prepared by A.O.A.C. Method **25.36(a)**.

COLLABORATORS COMMENTS

Rupert Hyatt.—N.F. Method, determination one, the precipitate was orange colored; No. 2 was lighter color and more of a yellow.

M. L. Dow.—The precipitate obtained in the modified N.F. VIII method was more granular and much more easily washed free of H_2SO_4 than the ppt. obtained following the N.F. VIII Method. The pressure bottles were kept in metal beakers as a precautionary measure.

J. A. Thomas.—As specified, the spirit of camphor was brought to 20°C. before taking aliquots. However, no attempt was made to bring the pipets to 20°C., as the high humidity in New Orleans would have caused condensation of moisture. With the pipet at 33°C., undoubtedly a volume change occurred when the camphor solution at 20°C. was transferred with the warm pipette.

DISCUSSION

The recovery of camphor by the modified N.F. VIII Method averaged 99.4 per cent and the maximum deviation from this value of any single determination was 0.6 per cent. As pointed out by Collaborator Thomas the measuring of a solution at 20°C. with a pipet at room temperature of 33°C. would cause a volume change which would be reflected in a slightly lower result. Collaborative work was done in the late spring and summer months.

Most of the camphor recoveries by the Official N.F. VIII Method were too low. Collaborators were requested to note the temperature of the refluxing condenser to determine how this factor affected recovery. While these varied from 18 to 30°C., the poorest yields were obtained at the two lower temperatures.

The Associate Referee believes that the following precautionary measure suggested by Collaborator Dow should be incorporated in the modified N.F. VIII Method under "Procedure," sentence 3, "close pressure bottle; immerse it in a covered metal beaker of water."

RECOMMENDATIONS*

It is recommended—

(1) That the proposed modification of the N.F. VIII Method be adopted as tentative.

(2) That the subject be closed.

No report was given on chemical methods for penicillin.

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* For report of Subcommittee B and action of the Association, see *This Journal*, **31**, 46 (1948).

REPORT ON SYNTHETIC DRUGS

By F. C. Sinton (Food and Drug Administration, Federal Security Agency, New York, N. Y.), *Referee*

For the past year 11 topics were assigned to the Referee. One additional topic was studied with a view to determining whether the present A.O.A.C method for phenolpthalein in chocolate preparations should be modified in certain details. Each topic and recommendation will be discussed separately.

RECOMMENDATIONS*

Quinacrine Hydrochloride.—A method which is shorter than the U.S.P. procedure has been recommended for adoption as an official method. It was further recommended that the topic be closed. The results obtained were good and the Referee recommends that the method be adopted as official, first action, and that further study be discontinued. The Associate Referee has left his position with the U. S. Food & Drug Administration but has expressed a desire to continue to serve the Association as Associate Referee.

Dihydrocodeinone.—The Associate Referee has submitted a report which includes a collaborative study. The results were generally close to the theoretical. The Associate Referee recommends adoption of the method as official, first action, and that the subject be closed.

Thiouracil.—A report was submitted and the Associate Referee has recommended that the method, which was studied collaboratively with reasonably concordant results, be adopted as tentative and that the topic be closed.

The Referee concurs.

Trichlorethylene.—An attempt was made by the Associate Referee to use the A.O.A.C. method for tetrachlorethylene. The results were not satisfactory and a further study was recommended by the Associate Referee.

The Referee concurs.

Carbromal.—Because of insufficient time the Associate Referee was unable to develop a method suitable for collaborative study. He has recommended that the study be continued.

The Referee concurs.

Butacaine Sulfate.—Some exploratory work was done on this problem but no formal report was submitted. The Associate Referee recommended that the subject be continued.

The Referee concurs.

Propadrine Hydrochloride.—The Associate Referee had no formal report, but has done some preliminary work. He experienced some difficulty in securing the pure salt for experimental use and recommends a continuance of the study.

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* For report of Subcommittee B and action of the Association, see *This Journal*, 31, 46 (1948).

The Referee concurs.

Methylene Blue.—The Associate Referee did some work on this topic but because of urgent regulatory work was unable to complete any conclusive determinations. Since methylene blue has been to a considerable extent replaced in medicine largely by the sulfa drugs and penicillin, the Referee recommends that this topic be dropped.

Sulfanilamide Derivatives.—No formal report was submitted, although the Associate Referee has undertaken a survey of the literature and has attempted the application of a number of methods. It was recommended that the subject be continued.

The Referee concurs.

Demerol.—The Associate Referee submitted collaborative results by the method previously published (*This Journal,* **28,** 711, 1945) and recommended that the method be advanced to official, first action, and the subject be closed.

Phenolpthalein in Chocolate Preparations.—Our attention was called to an article in *This Journal,* **26,** 194, 1943. A number of improvements over the present A.O.A.C. method were recommended. In order to investigate the matter an Associate Referee was appointed. He has conducted some experiments and finds merit in some of the proposals and recommends that the topic be continued with a view to collaborative study.

The Referee concurs.

Spectrophotometric Methods.—No report was received. It is recommended that the subject be continued.

No report was given on methylene blue, or on sulfanilimide derivatives.

REPORT ON ATABRINE (CHINACRIN, QUINACRINE)

By Harold C. Heim (Food and Drug Administration, Federal Security Agency, San Francisco, Calif.), *Associate Referee**

In a previous report[1] the Associate Referee reported the results of collaborative study of an extraction method for the assay of quinacrine hydrochloride and quinacrine hydrochloride tablets. This method has been found to yield slightly high results. As a topic for further collaborative study the Referee has suggested a method which has been in use for several years and which closely resembles the U.S.P. XII official method.

EXPERIMENTAL

A sample of U.S.P. XII quinacrine hydrochloride was assayed for total chlorine by a sodium peroxide bomb fusion with the following results:

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* Present address: Shool of Pharmacy, University of Colorado, Boulder, Colo.

[1] *This Journal,* **27,** 354 (1944).

TABLE 1.—*Results of chlorine analysis*

Cl FOUND	THEORY BASED ON ($C_{23}H_{30}OClN_3.2HCl.2H_2O$)	PURITY CALCULATED FROM Cl FOUND
per cent	*per cent*	*per cent*
20.62	20.90	98.66
20.71		99.09
Average		98.88

Samples of this quinacrine hydrochloride and a simulated tablet mixture consisting of quinacrine hydrochloride, talc, and starch were sent to collaborators with instructions, which are given with the method as published in *This Journal*, **31**, 115 (1948).

TABLE 2.—*Results with quinacrine hydrochloride*

ANALYST	FOUND	THEORY	RECOVERY (AVERAGE OF DUPLICATES)
	per cent	*per cent*	*per cent*
O. H. Miller	98.72; 98.89	98.88	99.9
M. L. Yakowitz	98.72; 98.69		99.8
A. G. Buell	98.69		99.8
R. D. Stanley	99.70; 99.95		100.9
Harry Isacoff	98.5 ; 98.6		99.7
H. C. Heim	98.68; 98.95		99.9
Average per cent recovery			100.0

TABLE 3.—*Results with simulated tablet mixture*

ANALYST	FOUND	THEORY	RECOVERY
	per cent	*per cent*	*per cent*
O. H. Miller	37.90; 37.93	37.95	99.9
M. L. Yakowitz	37.09; 37.45		98.2
A. G. Buell	37.67		99.3
R. D. Stanley	38.49; 38.43		101.3
Harry Isacoff	37.6; 37.7		99.2
H. C. Heim	37.43; 37.33		98.5
Average per cent recovery			99.4

The theoretical value for the simulated tablet mixture was calculated on the basis of a quinacrine hydrochloride of 98.88 per cent purity.

SUMMARY AND RECOMMENDATIONS*

(1) A method for the assay of quinacrine hydrochloride and quinacrine hydrochloride tablets has been subjected to collaborative study.

(2) Recoveries very closely approximating the theoretical values are included in this report.

(3) It is recommended that the method be adopted as official and that the topic be closed.

A REPORT ON THE COLLABORATIVE STUDY OF METHODS FOR THE DETERMINATION OF DEMEROL

By MERLIN MUNDELL† (Food and Drug Administration, Federal Security Agency), *Associate Referee*

Two methods for the determination of Demerol were reported[1] at the 1945 meeting of the A.O.A.C. At that time no collaborative work had been done on these methods. A sample was prepared for collaborative study which contained a mixture of Demerol hydrochloride, starch, lactose, and stearic acid; 102.0 grams of the mixture contained 17.0 grams of demerol hydrochloride. Samples were given to several collaborators who were asked to assay the samples by the two methods mentioned above. In printing the directions for the distillation method a typographical error was made. On page 712, line 12, of the paragraph headed "Determination" should have read "distill over a fourth 100 cc. portion of water." This correction was made in the copies of the methods sent to the collaborators.

Reports have been received from the following collaborators: Miss Muriel Drucker, New York Station, Food and Drug Administration, and Mr. Harold F. O'Keefe, Chicago Station, Food and Drug Administration. Their results are tabulated below.

TABLE 1.—*Per cent of demerol hydrochloride*

COLLABORATOR	BY DISTILLATION METHOD	BY EXTRACTION METHOD
#1	16.6	16.8
	16.7	16.7
	16.7	16.8
		16.7
#2	17.0	16.4
	17.0	16.5

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* For report of Subcommittee B and action of the Association, see *This Journal*, 31, 46 (1948).

† Present address, P.O. Box 793, Kilgore, Tex.

[1] *This Journal*, 28, 711 (1945).

The values obtained by the collaborators agree closely with the 16.67% Demerol Hydrochloride contained in the sample.

The following comments were made by Mr. O'Keefe:

> "I did not have the distillation set up described by you, so used instead the Hortvet type shown on page 186 of the *Book of Methods.*
>
> "I wonder if it might not be practicable to use a greater excess of N/50 H_2SO_4 as in routine laboratory work one cannot be certain of having 0.1 gm or less of the drug."
>
> "I don't see what advantage the standardization of the solution against sulfamic acid has over the official A.O.A.C. methods. I found that the factors obtained by the two methods checked very closely."

DISCUSSION

At the time the methods were presented the criticism was made that the accuracy of the method was not great enough to justify four significant figures in the factor for converting ml of 0.02 *N* sulfuric acid to g of demerol hydrochloride. It would therefore be better to use .00567 as the factor.

In regard to Mr. O'Keefe's comment that standardization of the solution against sulfamic acid has no advantage over the official A.O.A.C. methods, the Associate Referee feels that sulfamic acid has an advantage, in that it can be easily obtained in a pure form and can be maintained in the laboratory in dry form without change in strength. Standard solutions of the desired strength can be easily prepared from weighed samples. As Mr. O'Keefe pointed out, factors obtained by using sulfamic acid check very closely with the factors obtained by using the official A.O.A.C. methods. It would therefore be advisable to modify the wording of the methods to permit use of solutions standardized by the official A.O.A.C. methods.

Both the distillation and the extraction methods gave good results in the hands of the collaborators and no difficulties were reported. Details of the methods are published in *This Journal,* **31,** 115, 116 (1948).

RECOMMENDATIONS*

It is recommended that the methods, with the minor changes discussed above, be adopted as official, first action.

No report was given for propadrine hydrochloride; see report of Referee.

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* For report of Subcommittee B, and action of the Association, see *This Journal,* **31,** 46 (1948).

REPORT ON CARBROMAL

By RUPERT HYATT (Food and Drug Administration, Federal Security Agency, Cincinnati, Ohio), *Associate Referee*

Carbromal is official in the N.F. though no preparations containing it are listed and no assay is given. Some studies were made to find a suitable method for the assay of the crystalline material and commercial 5 grain tablets.

Three methods of assay were tried, *viz.*, extraction and weighing, nitrogen determination, and bromine determination. The extraction method gave low and variable results. The residue is appreciably volatile at 80°. This would make the drying of an extract difficult. The extraction method appears unsatisfactory.

The nitrogen determination appears promising. It would be necessary to use a separate portion for qualitative tests.

The bromine determinations were made by refluxing with alcoholic potassium hydroxide, the pressure bottle chloroform method, and charring methods similar to that for iodides (*Methods of Analysis*, 6th. Ed., 39.202). Slightly higher results were obtained by this method. The reason for this was not determined. No collaborative work was done.

It is recommended* that the subject be continued.

REPORT ON TRICHLOROETHYLENE

By GORDON SMITH (Food and Drug Administration, Federal Security Agency, New York, N. Y.), *Associate Referee*

Attempt has been made to use the existing method for tetrachloroethylene, with slight variation (*Methods of Analysis*, 6th Ed., p. 711). It is unlikely that chlorine can be quantitatively removed from a compound of this kind by any less drastic treatment than is there described. A sample of trichloroethylene purified by three distillations and having a density of 1.4556 25°/4° was used for the determinations.

Results have not been quite satisfactory. Recoveries between 99 per cent and 99.4 per cent have been obtained occasionally, but not consistently. Apparently slight differences in conditions can cause rather large differences in percentage recovery. Recently, recoveries over 98 per cent have been obtained consistently. It seems likely that with further study of details the method can be made to give results consistently close to 100 per cent, which will justify collaborative work.

Factors which appear to increase percentage recovery are:

1. Sodium not too finely divided.

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* For report of Subcommittee B and action of the Association, see *This Journal*, 31, 46 (1948).

2. Greater dilution with xylene (50 ml or over).
3. Fairly vigorous boiling.
4. Addition of amyl alcohol at the right intervals.

Further study is recommended.*

REPORT ON DIHYDROCODEINONE

By F. J. McNall (U. S. Food and Drug Administration, Federal Security Agency, Cincinnati, Ohio), *Associate Referee*

In accordance with the recommendation of Subcommittee B of the Association (*This Journal* **30**, 46), a method for the determination of dihydrocodeinone was subjected to collaborative study.

Dihydrocodeinone ($C_{18}H_{21}O_3N$), commonly known as Dicodid, is an alkaloid with a physiological action midway between codeine and morphine. It is used particularly as preliminary to operations to relieve postoperative pain, and is usually administered in the form of the bitartrate or hydrochloride.

Dicodid hydrochloride was selected as the material to be used for collaborative work and a 20% mixture of this substance with milk sugar as an excipient was submitted to collaborators to be assayed by the proposed method, details of which are given in *This Journal,* **31**, 117 (1948). The results reported by collaborators are as follows:

COLLABORATOR	DIHYDROCODEINONE HYDROCHLORIDE	COLLABORATOR	DIHYDROCODEINONE HYDROCHLORIDE
	per cent		*per cent*
1	19.98		
	19.91	5	19.26
			19.08
2	19.21		
	19.37	6	19.8
			20.2
3	20.52		
	20.60	Associate Referee	19.85
			20.35
4	19.5		
	19.7	Ave.	19.81

DISCUSSION

Dihydrocodeinone may be determined by the usual alkaloidal assay. The results of the collaborative samples are in good agreement, and no comments or criticisms were received regarding the proposed method.

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* For report of Subcommittee B and action of the Association, see *This Journal*, **31**, 46 (1948).

RECOMMENDATIONS*

It is recommended that the method for the determination of dihydrocodeinone be adopted as official (first action) and the subject be closed.

ACKNOWLEDGMENT

The Associate Referee wishes to thank the following persons, all members of the Food and Drug Administration, for their cooperation in this collaborative work: George E. Keppel, Minneapolis, Minn., H. P. Bennett Kansas City, Mo., Urban Oakdale and Wm. F. Kunke, Chicago, Ill., James H. McGee, St. Louis, Mo., and Rupert Hyatt, Cincinnati, Ohio.

No report was given on beutacaine sulfate; or on spectrophotometric methods.

REPORT ON THIOURACIL IN TABLET MIXTURE

By M. Drucker (Food and Drug Administration, Federal Security Agency, New York, N. Y.), *Associate Referee*

A study was undertaken to propose a determination for thiouracil in a tablet mixture.

Thiouracil powder was obtained with the cooperation of the manufacturer. When assayed, this sample was found to contain 98.7 per cent of the theoretical amount of nitrogen and 99.8 per cent of the theoretical amount of sulfur. The melting point of thiouracil (317°C. dec) cannot be used as a criterion of purity for routine laboratory work, because it is above the flash point of the common melting-point bath mixtures.

Thiouracil brominates very readily. Use of this fact was made in adapting a bromination procedure for the assay as reported in the literature.[1] Preliminary experiments served to indicate that the ease with which thiouracil reacts with bromine makes the assay an empirical method in which some conditions have to be specified. Several trial runs showed that about 0.125 g thiouracil and 25 ml of 0.5 *N* potassium bromide-bromate was a practicable combination. Using dried thiouracil powder, two additional sets of experiments were made by varying (Table 1) the bromination time and (Table 2) the quantity of thiouracil.

Experiment (A)

Conditions kept constant: 3 ml of 5% NaOH; 25 ml of 0.5 *N* $KBr\text{-}KBrO_3$; 10 ml of 10% HCl; 10 ml of 15% KI soln; reaction time with KI soln was exactly 5 minutes; titrated without the aid of starch.

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* For report of Subcommittee B and action of the Association, see *This Journal*, 31, 46 (1948).

[1] *J. Am. Med. Ass.*, 133, No. 9, 619 (1947).

TABLE 1.—*Results of varying time*

COLLABORATOR	WEIGHT OF THIOURACIL	EXACT BROMINATION TIME	CALCULATED THIOURACIL	PER CENT OF THEORETICAL
	gm	*min*	*gm*	*per cent*
1	0.1256	6	0.1221	97.2
2	0.1262	8	0.1244	98.6
3	0.1258	10	0.1238	98.4
4	0.1275	12	0.1264	99.1
5	0.1266	14	0.1269	100.2
6	0.1262	16	0.1270	100.6

Experiment (B)

Conditions kept constant: 3 ml of 5% NaOH; 25 ml 0.5 *N* KBr-$KBrO_3$; 10 ml of 10% HCl; bromination time was exactly 15 minutes; 10 ml of 15% KI soln; reaction time with KI soln was exactly 5 minutes; titrated without the aid of starch.

TABLE 2.—*Results with varying quantities of thiouracil*

	WEIGHT OF THIOURACIL	CALCULATED THIOURACIL	PER CENT OF THEORETICAL
	gm	*gm*	*per cent*
1	0.1000	0.0991	99.1
2	0.1104	0.1096	99.3
3	0.1206	0.1171	97.1
4	0.1300	0.1252	96.3
5	0.1406	0.1353	96.2
6	0.1505	0.1433	95.2

The foregoing results, as might be predicted, prove that the quantity of thiouracil combining with bromine increases as (1) the reaction time increases (Experiment A), and (2) the available amount of bromine increases (Experiment B).

A collaborative sample was prepared to resemble a typical tablet granulation. This was made from dried thiouracil, potato starch, talc U.S.P., lactose U.S.P., and stearic acid U.S.P., theoretically containing 50.00 per cent of thiouracil.

The collaborators, who are members of the Food and Drug Administration, were instructed to use a 0.5 g sample and to report the weight of sample taken. Their results are contained in Table 3.

COMMENTS

A. L. Diamond.—The only inconvenience found in the method was the length of time required to filter the gelatinous soln of thiouracil and alkali.

M. A. Braun.—I noticed that on addition of the NaOH the starch in the sample swelled and made filtration extremely difficult, making it necessary to change to a coarser filter paper. Even then the filtration was slow, and the filtrate was cloudy. Perhaps adding some water simultaneously with the NaOH would help some to prevent swelling of the starch.

TABLE 3.—*Thiouracil in tablet mixture No. 1*

COLLABORATOR	WEIGHT OF SAMPLE	PER CENT OF THIOURACIL FOUND	PER CENT OF THEORETICAL
	g		
A. L. Diamond, New York	0.5000	50.72	101.4
	0.5000	50.54	101.1
M. A. Braun, St. Louis	0.5024	51.1	102.2
E. H. Grant, Boston	0.5000	49.65	99.3
H. F. O'Keefe, Chicago	0.5006	51.17	102.3
	0.5008	50.71	101.4
Associate Referee	0.5056	49.84	99.7
	0.5029	49.59	99.2
	0.5026	49.34	98.7
Per Cent Average Recovery		50.29	100.6

TABLE 4.—*Thiouracil in tablet mixture No. 2*

COLLABORATOR	WEIGHT OF SAMPLE	PER CENT THIOURACIL FOUND	PER CENT OF THEORETICAL
	g		
M. A. Braun, St. Louis	0.5030	50.55	101.1
	0.5074	50.56	101.1
C. F. Bruening, Baltimore		52.05	104.1
		52.20	104.4
A. L. Diamond, New York	0.5000	51.35	102.7
	0.5000	51.30	102.6
H. F. O'Keefe, Chicago	0.5013	50.67	101.3
	0.4997	50.07	100.1
Associate Referee	0.5039	50.45	100.9
	0.5025	50.83	101.7
	0.5002	50.54	101.1
Per Cent Average Recovery		50.96	101.9

E. H. Grant.—By diluting almost to volume before adding the alkali and choosing a thin filter paper, I had no trouble with gel formation.

H. F. O'Keefe.—I took the first end point reached in the titration but noted that the iodine color returned to the material in a very short time.

DISCUSSION

Most of the collaborators experienced difficulty in filtering the alkaline solution of the tablet mixture. The Associate Referee washed the tablet powder down the sides of the volumetric flask with distilled water before adding the alkali and had no trouble with the analysis. She believed that the collaborators, in following the first set of directions, had weighed the dry powder into a volumetric flask and then had added alkali. Consequently, a second collaborative mixture, identical in composition with the first one, was prepared and sent to the original collaborators, together with directions which were modified ("Add 50 ml of distilled water and . . . ") by adding water to the weighed sample. Details of the method are published in *This Journal*, **31**, 117 (1948).

Collaborators' data are in Table 4.

COMMENTS

M. A. Braun.—No difficulties were encountered in following your procedure this time.

C. F. Bruening.—Starch was used as an indicator and it was noted that the blue color returned approximately one minute after the initial end point was obtained.

H. F. O'Keefe.—Your method was followed exactly as written but again I took the first end point reached, as the iodine color soon returned to the solution.

DISCUSSION

The collaborative work evidences that the empirical method studied in this paper provides a satisfactory assay for thiouracil in tablet mixtures.

It is recommended* that the method be adopted as tentative and the subject be closed.

REPORT ON PHENOLPHTHALEIN IN CHOCOLATE PREPARATIONS

By Harry Rogavitz (U. S. Food and Drug Administration, Federal Security Agency, New York, N. Y.), *Associate Referee*

A study was made to determine whether Hubacher's[1] modifications of the official method for phenolphthalein in chocolate preparations[2] should be incorporated into the A.O.A.C. method.

The A.O.A.C. method is based on the report of Palkin.[3] The collaborative sample prepared by Palkin consisted of 33⅓ per cent of phenolphthalein and 66⅔ per cent of cocoa. Some of the commercial preparations on the market contain about 10 per cent of phenolphthalein.

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* For report of Subcommittee B and action of the Association, see *This Journal*, **31**, 46 (1948).

[1] M. L. Hubacher, *This Journal*, **26**, 194 (1943).

[2] *Methods of Analysis, A.O.A.C.*, 6th Ed., sec. 39.168, p. 719.

[3] S. Palkin, *This Journal*, **8**, 541 (1924–25).

When a mixture containing 10 per cent phenolphthalein and 90 per cent of chocolate was analyzed by the A.O.A.C. method, low recoveries were obtained. The large-sized sample necessary for analysis of a 10 per cent phenolphthalein mixture results in considerable fat, which slows subsequent filtrations and entraps phenolphthalein. When the sample was analyzed by Hubacher's method, good recoveries were obtained and the time for analysis shortened. When this method was tried on a mixture of 10 per cent phenolphthalein and 90 per cent breakfast cocoa (high fat cocoa), recoveries greater than 100 per cent were obtained. When Hubacher's method was modified to include digestion of the tetraiodophenolphthalein precipitate on steam bath and washing with petroleum ether, recoveries approaching the theoretical amount were obtained.

It is recommended* that the subject be continued and that a collaborative study of the method be made.

REPORT ON MISCELLANEOUS DRUGS

By I. SCHURMAN (Food and Drug Administration, Federal Security Agency, Chicago 7, Ill.), *Referee*

RECOMMENDATIONS†

Compound Ointment of Benzoic Acid.—A report was submitted by the Associate Referee and he recommends that the method be adopted as tentative and the subject closed. The Referee concurs.

Iodine.—A re-investigation of the official method for Iodine (*Methods of Analysis*, 6th Ed. **39.202**). A report was received and the Associate Referee recommends that samples be submitted for collaborative study by the present official method and by the proposed modification of the official method. The Referee concurs.

Separation of Bromides, Chlorides, and Iodides.—The Associate Referee submitted a report covering a study by himself and Miss McMullen on Iodide, Bromide, and Chloride in Drugs, and the authors recommend:

(1) That the revised acetone method for chloride in the presence of bromide and iodide be subjected to further collaborative study.

(2) That the volumetric method for iodide and bromide be studied in comparison with the aeration absorption method and that one or both methods be submitted to collaborators.

(3) That the modified open Carius method as published (*This Journal*, **25**, 836, 1942) be adopted as a tentative method for total halides, with the notation, "Not applicable to many organic halogen compounds." Reagent (c) should be deleted from the list of reagents.

The Referee concurs.

The Associate Referee has requested that he be relieved of this assignment and recommends that the subject be reassigned. The Referee concurs.

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* For report of Subcommittee B and action of the Association, see *This Journal*, **31**, 46 (1948).

† For report of Subcommittee B and action of the Association, see *This Journal*, **31**, 47 (1948).

Preservatives and Bacteriostatic Agents in Ampul Solutions.—The Associate Referee has made a progress report and recommends that the subject be continued. Referee concurs in this recommendation.

Determination of Glycols and Related Products.—No report. The Associate Referee recommends continuance for another year. The Referee concurs.

Chemical Methods for the estimation of Estrone and Estradiol.—The Associate Referee reports that no samples were submitted for collaborative study and recommends that the subject be continued. The Referee concurs.

Chromatographic Separation of Drugs.—The Associate Referee reports that some preliminary work has been done, but not enough for a report. He recommends that the subject be continued. The Referee concurs.

Microchemical Tests for Alkaloids and Synthetics.—The Associate Referee has submitted a progress report in which he indicates that he has worked up considerable data on various compounds for inclusion in determinative tables for identification purposes and he recommends that the work be continued. He also recommends that the general title of the project be changed to "Microscopical Tests for Alkaloids and Synthetics" since this would include tests for both of a chemical microscopic nature and also crystallographic microscopic tests. The Referee concurs in both recommendations.

Study of the Ethanolamine Method for Mercurials, (including Phenyl Mercuric Acetate and Phenyl Mercuric Iodide).—The Associate Referee reports that work is under way and recommends that the subject be continued. The Referee concurs.

Phosphorus, Calcium, and Iron in Vitamin Preparations.—The Associate Referee reported that he has completed the preliminary work and is ready to submit sample for collaborative study, and recommends continuance. The Referee concurs.

Alkali Metals.—The Associate Referee has recommended that the subject be continued. The Referee concurs.

Organic Iodides.—No report received. The Referee recommends continuance.

Use of Superheated Steam in the Separation of Drugs.—No report. The Associate Referee has resigned from the Food and Drug Administration. The Referee recommends that the subject be dropped.

No report on microscopical tests for alkaloids and synthetics or on mercury compounds.

REPORT ON SEPARATION OF BROMIDES, CHLORIDES, AND IODIDES IN DRUGS

By **Newell E. Freeman** (*Associate Referee*) and **Beulah V. McMullen**, (Food and Drug Administration, Federal Security Agency, Atlanta, Ga.)

At the 1941 meeting (*This Journal*, **25**, 833) the Associate Referee reported on suggested methods for:

(1) Ashing with fixatives to eliminate interference of organic material. (Applicable in absence of iodide.)
(2) The "Open Carius" method for total halides.
(3) The recovery of halides as the sodium salts from the mixed silver precipitate. (Applicable in the presence of iodide.)
(4) The determination of iodide by an iron-aeration-KI absorption procedure.
(5) The determination of bromide by a ceric sulphate-aeration-KI absorption procedure.

Samples were prepared and sent out for collaborative study of points (2) and (3) above. These contained one, two, or three halides in the presence of sugar, glycerol, alcohol, and various organic drugs frequently associated with the halides in proprietary and official preparations. The collaborators were asked to determine total halide and to recover the halide from the silver salts by the methods given in the previous report. (*loc. cit.*, p. 836). In order to check the recovery of halides they were also asked to "Adjust the volume of the filtrate obtained by the hydroxylamine procedure to about 150 ml, acidify with ca 2 ml HNO_3 and add a slight excess of the $AgNO_3$ solution. Boil to agglomerate the silver halide, filter through a tared Gooch crucible and wash thoroughly with hot H_2O. Dry as before and weigh." Largely because of pressure of the war effort only a few results were obtained. These results are shown in Table 1.

The agreement between analysts on these five samples using the "Open Carius" method appears to be remarkably good and would indicate that the procedure, as outlined, is satisfactory for the determination of the three halides under consideration, either alone or in combination and in almost any mixture with organic substances where the halide itself is in the inorganic form. The hydroxylamine procedure appears to be quite satisfactory. The slight excess of silver salt recovered over that initially found, which is apparent in the majority of cases, may possibly be due to contamination by the reagents used. No blanks were run to correct for any halides present in the reagents. It is not clear why sample No. 5 shows a much poorer recovery than the others. All three halogens were present in this sample with only sugar and glycerine as organic matter. The fact that this precipitate was larger than any of the others, with the accompanying difficulty of obtaining complete disintegration and reduction of the precipitate, may be the explanation, in part, at least. It is believed that this procedure is sufficiently reliable to warrant its use in

TABLE 1.—*Weights silver halide obtained by "Open Carius" and by hydroxylamine recovery procedure*

METHOD	BRUENING	SCHIFFMAN	HAYNES	O'KEEFE	CONWAY
			Sample #1		
"Open Carius"	.5536	.5525	.5549	.5540	.5529
AgX/20 ml (1)	.5530	.5537			
Hydroxylamine	.5596	—	.5510		
Recovery g. AgX (2)	.5505	.5528			
			Sample #2		
1	.1104	.1128	.1127	.1110	.1115
	.1106	.1116	.1118		
2	.1117	.1148	.1170		
		.1133	.1122		
			Sample #3		
1	.1549	.1525	—	.1536	.1526
	.1558	.1528	—		
2	.1542	.1528	—		
	.1576	.1522	—		
			Sample #4		
1	.2127	.2119	.2120	.2110	.2122
	.2122	.2118	—		
2	.2112	.2130	.2028		
	.2116	.2122	—		
			Sample #5		
1	.6549	.6528	.6552	.6519	.6529
	.6543		.6561		
2	.6485	.6488	.6437		
	—		.6543		

those cases where the presence of iodide in the sample makes ashing procedures inapplicable.

DETERMINATION OF CHLORIDE IN THE PRESENCE OF LARGE AMOUNTS OF BROMIDE AND/OR IODIDE

Since the most usual case of mixed halides encountered in drug analysis is the presence of a small amount of chloride which enters the product as an impurity of the therapeutic agent or other ingredients, a method for this determination seemed desirable. This is particularly true with products containing large doses of bromides, since USP bromides may contain as much as 1 or 2 per cent of chloride (Cl^-), and the best reagent quality may contain as much as 0.2–0.5 per cent.

After preliminary trials, the method reported by McAlpine[1] (*cf.* Berg,[2]) was sent out, substantially as published, to a few collaborators with samples of USP and ACS bromides and iodides. The results reported are tabulated below:

TABLE 2.—*Chloride in presence of bromide and iodide*

COLLABORATOR	SAMPLE #1	SAMPLE #2	SAMPLE #3	SAMPLE #4
		(Mg chloride/3 gm)		*(Mg Cl/20 ml)*
Banes, Chicago	37.2			
	38.6	11.0		71.6
	38.1	17.1		72.8
Shuman, Phila.	25.2	10.8	1.5	49.6
	24.5	8.2	1.3	36.6
McClellan, N. O.	33.6	20.9	.12	57.2
	27.3	22.8	.27	82.0
	63.9	48.6		43.4
	31.2	71.7		49.4

In view of the difficulties encountered by some of the analysts and the erratic results obtained, modification of the method was attempted. This method depends on the reaction between acetone and free halogen to form halo-acetone according to the sense of the following series of equations:

$$10X^- + 2(MnO_4)^- + 16H^+ \rightarrow 5X_2 + 2Mn^{++} + 8H_2O \tag{1}$$

$$X_2 \rightleftarrows X^- + X^+ \tag{2}$$

$$X^+ + CH_3COCH_3 \rightleftarrows C_3H_5OX + H^+ \tag{3}$$

Since only half of the free halogen formed by reaction (1) is utilizable as X^+, more oxidant must be added so that from this standpoint equations (1) and (2) might be combined as:

$$X^- + 2 \oplus \cdots X^+ \tag{4}$$

Under the conditions here used Cl^- (chloride) is not affected and neither X^+ nor halo-acetone reacts with silver nitrate.

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[1] Mc Alpine, *J. Am. Chem. Soc.*, 51, 1065 (1929).
[2] Berg, *Z. Anal. Chem.*, 62, 342 (1926).

The principal difficulty with the procedure, as sent out for study, appears to be due to the reversibility of reactions (2) and (3), and to the fact that the excess of hydrogen peroxide added to destroy the excess potassium permanganate slowly reduces the X^+ to X^-. I^+ appears to be the more susceptible to this reduction. Decreased temperature and low concentration of both hydrogen peroxide and X^+ retard this reduction.

It has been found that, in the presence of ether, the chloride of silver is sufficiently agglomerated to permit filtration within one minute after the addition of the silver nitrate.

Consideration of equation (3) leads to the conclusion that a large excess of acetone and a low acidity would tend to retard the reverse reaction and so decrease the concentration of the X^+. However, too low an acidity slows up the potassium permanganate oxidation so that a practical balance must be drawn between these two desiderata. McAlpine (*loc. cit.*) calls for a solution 11 *N* with respect to sulfuric acid. Decreasing the amount of sulfuric acid so that the solution is 1.25–1.5 molar in this respect, gives much more satisfactory results. It not only retards the precipitation of silver bromide and silver iodide; but also greatly increases the oxidizing efficiency of the potassium permanganate with iodide and decreases the precipitation of manganese dioxide.

The details of the following method, which is the one now being used, have been worked out from the foregoing considerations; and it is believed that satisfactory results will be obtained by its use.

METHOD

CAUTION: The halogen acetone compounds produced in this procedure are extremely irritating to the eyes and respiratory tract. All operations should be performed under hood with good draft and care taken to avoid getting these vapors into the eyes or inhaling them.

Select a sample containing preferably not more than 1.5 milli-moles of Cl^- and not more than 5 g total halides. If organic matter is present, ash at dull red heat with an excess of calcium acetate.

Place sample in a 200–300 ml flask, add water to make a volume of ca 50 ml, 10 ml $MnSO_4$ soln (5 g/100 ml), and 20 ml dil. H_2SO_4(1+1). Add in small portions and with swirling to permit solution and reaction, an amount of solid $KMnO_4$ equal to 50–100 mg less than the quantity calculated to be necessary to convert all the Br^- and I^- to the free halogen; or, make a trial titration with 5% $KMnO_4$ soln and determine the amount of solid $KMnO_4$ to be used from this result. Cover the flask with a watch-glass, heat to boiling, and simmer slowly until most of the free halogen has been volitalized and only a light yellow color remains. Cool the soln, add ca 10 ml of acetone (free of reducing substances), dilute to 100 ml, and place in an ice-water bath. Add, dropwise, 5% $KMnO_4$ soln until the color of the soln changes thru amber to a deep wine or coffee color. Allow to remain in the ice bath about 15 min., adding more $KMnO_4$ from time to time, as the color fades.

Add 1% H_2O_2 soln, dropwise, slowly and with swirling to decolorize the soln and to dissolve any MnO_2 but avoiding any appreciable excess and keeping the liquid as cold as possible.

At once add 10 ml dil. HNO_3 (1+1), 10 ml ether, and then $AgNO_3$ soln (ca 0.1 *N*) with swirling until the opalescence decreases and the AgCl agglomerates, and then about 5 ml more (a total of 10–20 ml). Swirl ca 1 min and immediately filter thru a tared Gooch crucible. Wash the flask and ppt thoroly with water, then with a little alcohol, and finally with ether. Dry at 110°C., cool, and weigh.

$$1 \text{ g AgCl} = 0.2474 \text{ g Cl}^-$$

NOTE 1: A blank should be determined using all reagents, except the solid $KMnO_4$, in the amount used in the sample determination unless previous blanks on the same lot of chemicals have shown their chloride content to be negligible.

NOTE 2: If no opalescence appears in the filtrate within a half hour, add to it a few drops of $AgNO_3$. If a precipitate then forms, repeat the determination using more $AgNO_3$ or a smaller sample.

The following table gives the results obtained by the authors on the collaborative samples, using three modifications of this method, including the one just given in detail.

TABLE 3.—*Mg of chloride per 3 g sample*

SAMPLE NUMBER	METHOD AS SENT TO COLLABORATORS	SAME, BUT FILTERED AT ONCE	METHOD AS REPORTED HEREIN	(BVMo)
(1) USPNaBr	22.8	29.7	27.3	22.3
	23.5	22.3		
(2) ACS	5.4			
KBr	5.2	5.8	6.8	18.2
Reagent KI		0.8	0.1	
		2.9	0.3	0.2
			0.2	
(4) (0.2 g KI; 0.1 g NaCl)	69.8	90.6	69.6	72.5
	61.2			75.1

These results indicate a need for some further study of the procedure, especially where iodide is concerned.

Using the method here given, filtrates containing mono-brom-acetone in the presence of excesses of both silver nitrate and hydrogen peroxide remained perfectly clear for 2–3 hours. Those containing the iodo-compound precipitated much sooner, but even these did not show even a slight opalescence until about 20 minutes after filtration.

PRELIMINARY STUDIES OF A RAPID, VOLUMETRIC METHOD FOR THE DETERMINATION OF IODIDE AND BROMIDE IN THE PRESENCE OF CHLORIDE

The aeration methods previously reported upon seem to give satisfactory results, but they are time-consuming and require an all-glass aeration and absorption apparatus which may not be readily available to all analysts. Therefore the search for a rapid, simple method was continued. Various authors have studied volumetric applications of the oxidation of the halides to the +1 valent form (see equation 4 above). Among these, Andrews[3] used potassium iodate in strong hydrochloric acid solution to form iodine monochloride. Willard and Fenwick[4] used potassium permanganate in the presence of hydrocyanic acid to oxidize bromide to bromine cyanide with electrometric determination of the end point. Kolthoff[5] and Lang[6], using potassium iodate as oxidant, and Berg[7], using potassium bromate, have studied this halogen-cyanide reaction extensively. Since it appeared most promising, this reaction was selected for study in the present investigation. The method depends upon the oxidation of iodide and bromide in the presence of hydrocyanic acid according to equation (4) above and the following reaction:

$$\text{(5.)} \qquad X^+ + HCN \rightleftarrows XCN + H^+$$

During the oxidation, when both X^- and X^+ ions are present, equation 2 becomes evident. This, and the fact that Br^- is not effected as long as I^- is present, are utilized for the titration of I^-. As in the Andrews method (*loc. cit.*) chloroform (or in this case starch) is added at the beginning of the titration and the oxidant is added up to a disappearance of the iodine color.

The halogen cyanides appear to be more stable and the X^+ ions less readily reduced than is the case with the acetone compounds. Lang, Kolthoff, and Berg (*loc. cit.*) mention a large number of reducing agents which may be used to remove excess oxidant from the reaction mixture without affecting the halogen cyanide. These include hydrazine, phenol, aniline, oxalate, and ferrous ions.

In order to complete the oxidation of Br^- to bromine cyanide, a slight excess of oxidant is required, as well as a somewhat increased acidity. Since no visual indicator for this equivalence point has been found, the removal of the excess oxidant becomes essential. Then, when all the I^- and Br^- have been converted into the corresponding cyanides, the reverse of reactions (5) and (2) are utilized by adding potassium iodide and titrating the liberated iodine with standard thiosulfate.

$$\text{(6.)} \qquad 2XCN + 21^- + 2H^+ \rightarrow I_2 + 2X^- + 2HCN$$

Chloride requires a very large excess of oxidant and a very high acid concentration before it is oxidized to cyanogen chloride and therefore does not interfere with the determination of iodide and bromide.

The principal difficulty found in applying the methods of Lang and Berg lay in the removal of the excess $(IO_3)^-$ or $(BrO_3)^-$ without some of the halogen from these compounds being captured, *en passent*, by the hydrocyanic acid. Using various reducing agents recommended by the aforementioned authors, residual oxidation blanks varying between .05 and .3 ml. of 0.1 *N* thiosulfate per ml of *M*/40 oxidant were obtained. Ferrous salts were found to be unsatisfactory for the purpose, not because they reduced the X^+ ions, but because the ferric ion formed in reducing the excess oxidant, itself oxidized some of the I^- to I_2 in the highly acid solutions used (ca 2*N*).

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[3] Andrews, *J. Am. Chem. Soc.*, 25, 756 (1903); *cf.* USP XIII, 273.
[4] Willard and Fenwick, *Ibid.*, 45, 623 (1923).
[5] Kolthoff, *Mikrochemie*, 3, 75 (1925).
[6] Lang, *Z. anor. allgem. Chem.*, 122, 332 (1922); 142, 229, 279 (1925); 144, 75 (1925).
[7] Berg, *Z. Anal. Chem.*, 69, 1, 369 (1926).

Cerate oxidation was tried in order to eliminate halogen-containing oxidants and permanganate, which is notorious for its action on chloride. Cerate was found to be quite satisfactory after some trials and adjustments were made. The sulfuric acid solution of the USP (ca *M*/2 in sulfuric acid) reacted much too slowly with Br^- and this was still true when the sulfuric acid contents of both standard and solution being oxidized were increased to ca 2.5 *M*. However, in nitric acid solutions, 1.5 –2 *M* in nitric acid, bromide was completely oxidized at a temperature of ca 45°C in 15–30 minutes. Nitric acid solutions of this strength have no effect on I^- even over considerable periods of time, provided that no nitrous acid or oxides of nitrogen are present. This can be accomplished by boiling the nitric acid down with a little water and then diluting with an equal volume of water.

Potassium iodide solutions titrated to iodine cyanide with cerate and then treated with an excess of the oxidant at 45°C for 30 minutes showed no appreciable increase in oxidizing value after reduction of the excess cerate.

0.3 g of sodium chloride treated in the same way showed a titration of only 0.1 ml N/10; 3 g sodium chloride in the presence of 0.1 g potassium bromide showed no appreciable difference in titration from 0.1 g potassium bromide treated in the same way with no Cl^- present.

In order to check the effect of different reducing agents on the halogen cyanide, a stock solution of bromine cyanide was prepared by oxidizing a quantity of reagent potassium bromide with the least possible excess of potassium permanganate and removing this excess with a minimum amount of hydrogen peroxide. After standing overnight, excesses of the reducing agents were added to identical portions of this stock solution, and the resulting mixtures were allowed to stand 15–30 minutes before adding potassium iodide and titrating. Among the substances tried were hydroxylamine, hydrazine, oxalate, hydrogen peroxide, and ferrous sulfate. The aromatic amines and phenols were not tried because of the colors produced by their oxidation. Of these, hydroxylamine proved far the most satisfactory. Two millimoles of this reagent added to the titration mixture containing bromine cyanide, produced, after 30 minutes, a decrease in titration of only 0.2 ml 0.1 *N* from that obtained on a similar solution to which no reducing agent had been added.

Hydroxylamine is also a convenient reducing agent to use in other phases of this investigation since an excess of this substance can readily be eliminated, without affecting the state of other components, by boiling with excess sodium hydroxide, especially in the presence of a little alcohol. For example, 10 millimoles of hydroxylamine (as the sulfate) were added to a sodium carbonate solution of ca 0.2 g potassium iodide and a drop or two of bromine water. The solution was boiled for a few minutes and then 2 ml of saturated sodium hydroxide solution and 2 ml of alcohol were added and the boiling was continued for 10 minutes. After cooling and acidifying, an amount of free iodine equivalent to 0.25 ml 0.1 *N* was found. This small blank can probably be eliminated by protecting the alkaline solution from oxidation by the air.

This fact forms the connecting link between the hydroxylamine recovery procedure referred to in the first part of this paper, and the volumetric method here considered. It is thus possible to use the open Carius silver halide isolation and the hydroxylamine recovery procedure as preliminary preparations for the volumetric determination of iodide and bromide, in those cases where the presence of both iodide and organic matter make them necessary. They should also be used in the presence of iron, sulfur compounds, arsenic, or other inorganic oxidizing or reducing substances.

The method in outline form is as follows:

The cerate soln contains 63.5 g of ceric ammonium sulfate $(NH_4)_4Ce(SO_4)_4 \cdot 2H_2O$ dissolved in 200 ml dilute HNO_3(1+1) and enough water to make 1 liter.

The sample should contain from 0.5–2 millimoles of either I^- or Br^- and, preferably, not more than 2 millimoles total.

Iodine present.—In a volume of ca 70 ml, add 10 ml KCN soln (6.5 g/100 ml) and enough dilute nitric acid (1+1) to equal 20 ml per 100 ml. Add starch and titrate with standard cerate soln to the disappearance of the starch-iodine color.

Bromide present.—Use the soln titrated for iodide or add potassium cyanide and dilute nitric acid as in the preceding paragraph. Add the cerate soln in slight excess (the yellow color of the cerate is evident) and warm at 45°C. for 30 min. Cool, discharge the excess cerate with $NH_2OH \cdot H_2SO_4$ soln (8.2 g/100 ml). Add potassium iodide and titrate with 0.1 *N* sodium thiosulfate, adding starch near the end point if necessary.

The difference between the thiosulfate titration and the cerate titration for iodide is that due to bromide.

SUMMARY

Very satisfactory results are reported of the collaborative study of the modified open Carius method for total halides and of the hydroxylamine procedure for the recovery of the halides from the mixed silver precipitate. (Both previously published, *This Journal*, 25, 836, 1942.)

A method for the determination of small amounts of chloride in the presence of large quantities of iodide and bromide was submitted to collaborative study. The results were very erratic and erroneous, but the method has now been revised and the procedure reported herein is believed to be reliable.

A rapid volumetric method for the determination of iodide and bromide in the presence of chloride has been studied on a preliminary basis and appears to hold much promise.

RECOMMENDATIONS*

It is recommended—

(1) That the revised acetone method for chloride in the presence of bromide and iodide be subjected to further collaborative study.

(2) That the volumetric method for iodide and bromide be studied in comparison with the aeration absorption method and that one or both methods be submitted to collaborators.

(3) That the modified open Carius method as published (*This Journal*, **25**, 836, 1942) be adopted as a tentative method for total halides, with the notation, "Not applicable to many organic halogen compounds."

Reagent (C) should be deleted from the list of reagents.

No report was given on organic iodides.

REPORT ON COMPOUND OINTMENT OF BENZOIC ACID[1]

By WILLIAM F. KUNKE (Food and Drug Administration, Federal Security Agency, Chicago, Ill.), *Associate Referee*

Collaborative work was undertaken this year as recommended by Subcommittee B, *This Journal*, **28**, 52 (1945). None has been done previously.

A brief review of the previous work by the Associate Referee has been reported. *This Journal*, **28**, 723 (1945). This included an experimental study of the quantitative bromination of salicylic acid, which was done because the literature is surprisingly meager in giving thorough and critical investigations. The most important experiments are given in Table 1, to show what the optimum conditions are, and how certain deviations from those conditions affect the quantitative results.

By the proposed method[2] the benzoic and salicylic acids are extracted together and titrated with 0.1 *N* sodium hydroxide; and after evaporation of the solvent (alcohol) the salicylic acid is determined bromometrically (benzoic acid does not consume bromine) and the benzoic acid is calculated by difference.

COLLABORATIVE SAMPLES

The benzoic acid and salicylic acid were separately and accurately weighed directly in a small vial and the approximately equivalent molten

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* For report of Subcommittee B and action of the Association, see *This Journal*, **31**, 47 (1948).

[1] National Formulary VIII: Ointment of Benzoic and Salicylic Acids.

[2] The details of the method are published in *This Journal*, **31**, 117 (1948).

TABLE 1.—*Bromination of salicyclic acid**

DETERMINATIONS	Br_2 IN EXCESS	HCl	REACTION PERIOD	SALICYLIC	
				MIN.	MAX.
	per cent	*ml*	*min.*	*per cent*	*per cent*
20	25	5	30	99.8	100.2
3	25	5	60	100.0	100.3
6	25	10	30	99.4	99.8
4	25	10	45	98.5	99.0
7	25	5	150	101.3	101.7
11	100	5	30	100.1	101.0
4	25	20	30	92.4	97.3
1	25	5	10	97.9	—
2	25	10	10	98.6	98.8
2	25	20	10	88.4	92.8
1	1.3	5	30	97.3	—
1	1.3	12.5	60	95.0	—
2	1.3	5.0	1	76.8	91.5

* Total volume in each determination 100 ml.

quantity of the (previously thoroughly mixed) ointment base was added. The collaborator was requested to use the entire contents of one vial for a determination. The preparation of the collaborative samples in this manner avoided the possibility of non-uniformity of a sample sufficiently large for all the collaborative work. It is well-known that an ointment of uniform consistency is not readily prepared by hand; furthermore, an increase in temperature will cause partial segregation.

According to the National Formulary VIII the ointment contains 12 per cent of benzoic acid and 6 per cent of salicylic acid incorporated in a white petrolatum and wool-fat base.

TABLE 2.—*Collaborative results*

ANALYST	BENZOIC ACID		SALICYLIC ACID	
	gram	*per cent*	*gram*	*per cent*
Conroy	0.2962	99.3	0.1589	99.5
	0.2952	99.0	0.1593	99.8
O'Keefe	0.2926	98.1	0.1580	99.0
	0.2924	98.1	0.1588	99.5
McNair	0.2911	97.6	0.1606	100.5
	0.2904	97.4	0.1614	101.0
	0.2906	97.4	0.1592	99.7
Fine	0.2895	97.1	0.1617	101.2
	0.2932	98.0	0.1604	100.4
Hyatt	0.289	97.0	0.157	98.3
	0.292	98.0	0.157	98.3

RECOMMENDATIONS*

It is recommended—

(1) That the proposed method be adopted as tentative.

(2) That no further work be done.

No reports were given on alkali metals or on glycols and related compounds.

REPORT ON PRESERVATIVES AND BACTERIOSTATIC AGENTS IN AMPUL SOLUTIONS

By CHARLES N. JONES (Food and Drug Administration, Federal Security Agency, New York, N. Y.), *Associate Referee*

The recommendations of the previous report[1] on this topic were followed in that attempts were made to find some qualitative method for the detection of the mercurial type of preservative. In place of having individual tests for the several preservatives, the problem resolved itself into the detection of a small amount of organic mercury. One method was tried with considerable promise, using dithizone as the ultimate reagent for mercury. However, this method was not studied objectively enough to justify its incorporation into this report.

It is recommended* by the Associate Referee that the work be continued.

No report was given on phosphorus, calcium, and iron in vitamin preparations; see report of Referee on miscellaneous drugs.

REPORT ON IODINE

By SAM D. FINE (Food and Drug Administration, Federal Security Agency, Cincinnati, Ohio), *Associate Referee*

The present official method for iodine[2] gives low results on samples which contain small amounts of organic material. The loss of iodine occurs in such samples at the point where the procedure directs " . . . Neutralize filtrate with sulfuric acid (1+1), make alkaline again with 4 per cent sodium hydroxide solution, and add 1 ml. in excess . . . ." It was noted that free iodine was liberated as the sulfuric acid was added to the strongly alkaline solution. It was evident that during the charring some iodate must have been formed which reacted with the remaining iodide to liberate iodine.

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* For the report of Subcommittee B and action of the Association, see *This Journal*, 31, 47 (1948).

[1] *This Journal*, 30, 486 (1947).

[2] *Methods of Analysis*, 6th Ed., sec. 39.202, page 728.

EXPERIMENTAL

The Associate Referee tried two different procedures in order to obtain higher recoveries. The first was to omit the step beginning with "... Neutralize filtrate with sulfuric acid (1+1)..." and proceed with the oxidation with potassium permanganate in the strongly alkaline solution, instead of in the slightly alkaline solution specified. A potassium iodide solution of known concentration was assayed by the method as written and by the modification above. Recoveries were 92.8 and 94.5 per cent, respectively.

The second procedure was to add organic material (sodium benzoate) to the potassium iodide solution before charring. The solution, plus organic material, was assayed by the method as written and by the modification above. Recoveries were 98.4 and 96.3 per cent respectively.

All of the above determinations were charred in a furnace simultaneously at 500°C. and for the same length of time.

The following additional experiments were conducted:

(1) A known amount of potassium iodide and 3 g of potassium hydroxide were dissolved in sufficient water to equal the volume normally obtained in the filtrate from the charred mixture; and then the procedure specified in the official method was followed. Recovery was 99.95%.

(2) A known amount of potassium iodide and 3 g of potassium hydroxide were charred and the procedure specified in the official method was followed. The alkaline filtrate was chilled in an ice-bath and the sulfuric acid was added very slowly. Iodine color was noted in the soln on the addition of sulfuric acid. Recovery was 96.63%.

(3) A known amount of potassium iodide, 1 g of sodium benzoate, and 3 g of potassium hydroxide were charred at the same time as in (2) and the procedure specified in the official method was followed. There was no evidence of iodine color on the addition of the sulfuric acid. Recovery was 99.40%.

DISCUSSION

These experiments demonstrate that the low results obtained by the present official method on samples which contain small amounts of organic material can be corrected. The best way to correct these low recoveries seems to be by the addition of organic material to the sample before charring, which prevents the formation of iodate during the charring, with resultant loss of iodine when sulfuric acid is added. Direct oxidation in strongly alkaline solution results in only partial improvement of the recovery on samples of this nature.

It is recommended* that samples be submitted for collaborative study with instructions to make determinations by the present official method

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* For report of Subcommittee B and action of the Association, see *This Journal*, 31, 47 (1948).

and by the proposed modified official method (addition of organic material before charring).

No reports were given on superheated steam in separation of drugs, on estrone and estradiol, or on chromatographic separation of drugs.

There was no report on drug bioassays, or an any of the included subjects.

REPORT ON SPECTROGRAPHIC METHODS

By W. T. Mathis (Agricultural Experiment
Station, New Haven, Conn.), *Referee*

No attempt has been made so far to enlist collaboration on spectrographic methods for the reason that, with the exception of analysis of plant materials, the work in our laboratory has been mainly exploratory, in an effort to find the most promising possibilities for use of the spectrograph on general analytical problems.

Some merely interesting, and some very good, results were obtained on many materials, such as ammonium acetate soil extracts for major and minor constituents; spray residue extracts for lead, copper, mercury; urine for lead; and biological and miscellaneous samples for semi-quantitative estimation of poisonous metals, etc. Much additional work will be required, however, to bring some of the procedures up to the point where they are ready for collaborative trial.

It would seem that some standardized procedure of electrode preparation, excitation, etc., could be made to cover a large portion of such analyses; and in this connection present quantitative technique employed in our laboratory is outlined and suggested for consideration. This technique, applicable to the determination of both major and minor constituents, was selected on the basis of better uniformity of results over other schemes that were tried.

Craters 5 mm in diameter and 6 mm deep are drilled in purified ¼″ graphite electrodes and are packed with the graphite removed in the drilling process. Solutions to be analyzed are added to the electrodes and allowed to soak into the packed carbon and dried in an oven.

Alternative preparation, when salts are extremely high, is to treat a weighed portion of the carbon drillings with a definite amount of the sample solution. The mixture is dried, mixed thoroughly, and a portion packed into the electrode crater.

High voltage A.C. spark, with inductance, is used for excitation, with the opposing electrode pointed. During a sparking time of 25 seconds the impregnated carbon is sprayed from the crater, the particles burning in such a way that distillation variables encountered with D.C. arc excitation are apparently eliminated.

Cobalt is used as an internal standard and all intensity ratios are based on a single cobalt line, 3044 Å. On this basis the technique becomes applicable to many types of agricultural materials and in many cases the same analysis scale values may be used.

As some analyses are on the basis of "available" constituents, and some are on surface extracts, etc., we have followed wet preparation of all samples for the sake of uniformity in leading up to what we like to visualize as a reasonably "universal technique." Sample preparation in general is as follows:

Plant materials are ashed, dissolved in dilute hydrochloric acid, and evaporated to dryness.

Soil extracts are evaporated to dryness, etc.

Aliquots of spray residue extracts are evaporated to dryness.

Miscellaneous materials are treated appropriately and their solutions evaporated to dryness.

The salt residues from the above treatments are dissolved by warming with definite volumes of acid-cobalt solution, containing 150 ml of hydrochloric acid and 200 mg of cobalt per liter. Tenth milliliter portions are added to the prepared electrodes in duplicate and dried at 130° C.

Obviously, desired concentrations may easily be obtained by varying either the amount of sample or the amount of acid-cobalt solution. Our analysis scales (in per cent) are based on 1 gram of sample in 5 ml acid-cobalt solution, the usual concentration for plant material. In using the same analysis scales for soil extracts, where concentration of 20 grams in 1 ml acid-cobalt solution is desirable, the 1.0 per cent value on the analysis scale becomes 0.01 per cent, etc.

The acid-cobalt solution may also contain sodium or lithium if desired, to overcome the effect on other elements of extreme variations in major constituents of samples. An alternative to the use of such salt buffers is to determine these effects from appropriate standards and use correspondingly corrected analysis scales for the varying ranges of major constituents. This latter scheme has worked to a reasonably satisfactory degree in our laboratory, and has certain practical advantages over the use of salt buffers, particularly with regard to plant materials.

During the past year several hundred pasture grass and tobacco samples were analyzed both spectrographically and chemically in our respective laboratories, with very gratifying agreement in results. The determinations involved were potassium, calcium, magnesium, phosphorus, and manganese, and in almost every instance of an occasional original disagreement a second chemical result checked the spectrograph. On many samples additional determinations of iron, aluminum, zinc, sodium, copper, and boron were made spectrographically but not chemically.

As a typical example of uniformity between duplicate electrodes as run by this technique, we cite the following analyses of twelve tobacco samples recently run in our laboratory:

TABLE 1.—*Results on 12 tobacco samples*

	K	Ca	Mg	P	Mn	Fe
Percentage Range	4.04–5.54	3.50–4.15	.43–.80	.29–.40	.012–.027	.051–.139
Maximum deviation from average	.26	.18	.04	.015	.001	.005
in % of amount present	4.7	4.7	8.3	4.1	3.7	5.1
Average deviation from average	.10	.05	.015	.004	.0002	.0026
in % of average amount present	2.2	1.3	2.6	1.2	1.1	2.6

It would appear that this accuracy of *duplication* is reasonably within the limits of the average routine chemical work. The actual correctness of results, however, depends upon the standardization for the particular material.

This standardization for all types of plant material is suggested as an important subject for collaborative study, particularly if a technique is used which may be applied to extracts and materials in general. The information gained will then assume a general significance which will be very helpful in working with miscellaneous agricultural materials. It is probable that the following subjects, or at least some phases of them, will fit very nicely into this picture:

Soil extracts for available constituents.
Spray residues.
Foods and biological materials.
Fertilizer materials.

RECOMMENDATIONS*

It is recommended—

(1) That analysis of proper samples of various types of plant materials be made by collaborators using their own techniques, so that the most promising procedures may be indicated for further study and trial by all collaborators.

(2) That any subjects or determinations of particular interest, to which the spectrograph might be applied, be suggested for study.

No report was given on radioactivity (quantum counter).

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* For report of Subcommittee B and action of the Association, see *This Journal*, 31, 45 (1948).

TUESDAY—AFTERNOON SESSION

REPORT ON MICROCHEMICAL METHODS

By C. O. WILLITS (*Referee*) AND C. L. OGG (Eastern Regional Research Laboratory* Philadelphia 18, Pa.)

There has been no report on microchemical methods since the one made in 1941 by the late E. P. Clark. Inasmuch as the recognition of microanalytical methods has steadily increased it is desirable that referee work leading to the standardization of these methods be resumed.

Since about forty quantitative microchemical methods are now in use, it seemed too arbitrary for the Referees to select the methods for study or to designate the methods to be studied first. Few, if any, of the laboratories equipped to perform microanalyses employ all the micro methods. It was not known what determinations each laboratory was making or was prepared to make, nor was it known which laboratories would be willing to participate in collaborative studies on microchemical methods. Consequently, a questionnaire was sent to microchemists throughout the country asking for their opinions on the following questions: (1) Should standardization of microchemical methods be attempted? (2) Which determinations should be standardized? (3) Which determinations should receive first attention? Also in the questionnaire were the questions: (1) Would you participate in collaborative studies? (2) What determinations are you performing in your laboratory?

More than seventy-three replies to the questionnaire have been received, and returns are still not complete. The replies have clearly shown that collaborative work on standardization of micro methods is endorsed by the microanalysts throughout the country. Of the seventy-three microchemists who replied, none were opposed to the studies, and only three were indifferent. Many not only answered the questionnaire but also endorsed the proposed work. The response to the question regarding willingness to cooperate in these studies has also been gratifying. Forty-seven expressed a desire to participate, and most of the twenty-six unable to collaborate stated that they were not able to do so because of the nature of their present work or lack of equipment or personnel.

To determine the geographic location of the microanalytical laboratories in this country, the replies received from the East, Midwest, and West were tabulated, with the following results: 70 per cent are in the East, 20 per cent in the Midwest, and 10 per cent on the West Coast.

The replies to the question regarding methods that should be standardized mentioned eighteen in addition to the eighteen listed in the questionnaire, making a total of thirty-six suggested. The ten determinations that received the most votes for standardization were: Carbon and hydrogen (37), sulfur (37), Kjeldahl nitrogen (36), molecular weight (36), Dumas nitrogen (35), chlorine (30), bromine (27), iodine (26), acyl group (25), alkoxyl group (25). These were closely followed by phosphorus, hydroxyl group, neutralization equivalent, and oxygen. The remainder received 18 or fewer votes. A large percentage of the chemists who regularly perform a determination recommended standardization of the method.

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* One of the Laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture.

The selections of methods to be studied first, second, third, and fourth were weighted on a point system by multiplying each first choice by 4, second by 3, third by 2, and fourth by 1. The ten determinations which received the largest number of points were: Carbon and hydrogen (114), Dumas nitrogen (69), Kjeldahl nitrogen (56), sulfur (39), chlorine (33), molecular weight (31), oxygen (19), bromine (13), iodine (12), and acyl groups (11).

In view of these replies to the questionnaire, the Referee and Associate Referee recommend† that collaborative studies be initiated on the methods for the determination of carbon and hydrogen, and the Dumas and Kjeldahl methods, for the determination of nitrogen.

REPORT ON STANDARD SOLUTIONS

By H. G. UNDERWOOD (Food and Drug Administration, Federal Security Agency, Chicago, Ill.), *Referee*

Standard Sodium Thiosulfate Solutions.—The method for the standardization of sodium thiosulfate solutions was adopted as official, first action, in 1945, on the basis of excellent collaborative results, and after the Associate Referee had demonstrated that the solutions are stable for long periods of time under practical conditions. It was decided in 1945 that further studies were unnecessary. The method has been in use for a sufficient period of time to warrant its adoption as official, final action.

Thiocyanate Solutions.—No formal report has been received on this subject in view of the recommendation last year that the study be closed. The method should be adopted as official, final action.

Potassium Permanganate Solutions.—After the Referee on synthetic drugs observed that the official A.O.A.C. procedure for standardizing potassium permanganate solutions yielded higher results than the procedure recommended by the National Bureau of Standards, this subject was reopened. In 1944, Mr. Duggan found that the titers obtained by the A.O.A.C. procedure were 0.2–0.4 per cent higher than were obtained by that recommended by the Bureau of Standards. During the past year Mr. Duggan and two collaborators confirmed this difference and found that the results obtained by the Bureau of Standards procedure compare favorably with those obtained by direct comparison with Bureau of Standards arsenious oxide, and by indirect comparison with Bureau of Standards potassium dichromate. Although until this year Mr. Duggan did not favor changing the A.O.A.C. method, he is now convinced that the method which is now official should be replaced by that recommended by the Bureau of Standards. On the basis of the additional data, the method recommended by the Bureau of Standards should be adopted as official, first action.

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† For report of Subcommittee C and action of the Association, see *This Journal*, **31**, 53 (1948).

Bromide-Bromate Solutions.—Mr. Van Dame submitted to collaborative study the procedure reported in 1946 for the standardization of bromide-bromate solutions against Bureau of Standards arsenious oxide. The maximum variation from the average of 35 determinations made by seven collaborators was 2 parts in 1000, and that in only 2 determinations. On the basis of these results, the method should be adopted as official, first action, and the study closed.

Titanium Trichloride Solutions.—Miss Breit has continued the study of the three methods of standardization of titanium trichloride solutions reported on last year. On the basis of the results obtained by Miss Breit and one collaborator in the same laboratory, and because of the simpler procedure, Miss Breit favors the method of standardization by comparison with Bureau of Standards potassium dichromate, using diphenylamine as indicator; she recommends that the method be adopted as tentative. However, since titanium trichloride solutions are not stable and are difficult to handle, it is believed that collaborative studies should be made in more than one laboratory before adopting any one of the methods as tentative.

Potassium Dichromate Solutions.—No report was received on the subject. This study was recently re-assigned to Mr. McClellan of the Food and Drug Administration, New Orleans, La.

Standard Buffer Solutions.—Dr. Manov reports the preparation of larger quantities of pure buffer salts for certification by the National Bureau of Standards. He proposes to submit samples of standard buffer salts and unknown buffer solutions to a few laboratories for collaborative *p*H measurements. This subject should be continued.

RECOMMENDATIONS*

It is recommended—

(1) That the method for the preparation and standardization of sodium thiosulfate solutions described in *This Journal*, **25**, 659 (1942) be adopted as official, final action;

(2) That the method recommended by the National Bureau of Standards for the standardization of potassium permanganate solutions be

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* For report of Subcommittee A and action of the Association, see *This Journal*, **31**, 44 (1948).

adopted as official, first action, and that it replace the official method appearing in the Sixth Edition of the Methods of Analysis, p. 807.

(3) That the method for the preparation and standardization of bromide-bromate solutions be adopted as official, first action;

(4) That the methods for standardizing titanium trichloride solutions be studied further;

(5) That the study of the preparation and standardization of potassium dichromate solutions be continued;

(6) That the studies on buffer solutions be continued;

(7) That the method for the preparation and standardization of thiocyanate solutions be adopted as official, final action.

No report was made on potassium dichromate solutions, or on thiocyanate solutions.

REPORT ON STANDARDIZATION OF POTASSIUM PERMANGANATE SOLUTIONS

By R. E. DUGGAN (Food and Drug Administration, Federal Security Agency, New Orleans, La.), *Associate Referee*

In previous reports (1, 2) on the comparison of the official A.O.A.C. procedure (3) and the procedure of Fowler and Bright (4), recommended by the National Bureau of Standards, for the standardization of potassium permanganate solutions using sodium oxalate as the primary standard, the Associate Referee has stated that the titers obtained by the A.O.A.C. procedure are 0.2–0.4 per cent higher than titers obtained by the Fowler and Bright procedure, which, for the sake of convenience, will be referred to as the National Bureau of Standards procedure. The two procedures are as follows:

PROCEDURE I—A.O.A.C.

Weigh 0.25–0.30 g of Bureau of Standards $Na_2C_2O_4$ in sufficient H_2O to make soln ca 0.1 *N*. Add 15 ml of 4 N H_2SO_4 for each 50 ml of soln. Heat of 75–85° and titrate with the $KMnO_4$ soln, maintaining this temp. thruout titration. Add the $KMnO_4$ slowly, especially at beginning, and wait each time until soln becomes colorless. Continue titration to end point, with continuous stirring. Correct for excess of $KMnO_4$ used for end point by matching color in another beaker containing same quantity of acid and hot H_2O.

PROCEDURE II—N.B.S.

Transfer 0.3 g of sodium oxalate (dried at 105°C.) to a 600-ml beaker. Add 250 ml of diluted sulphuric acid (5+95) previously boiled for 10–15 min. and then cooled to 27±3°C.

Stir until the oxalate has dissolved. Add 39 to 40 ml of 0.1 *N* potassium permanganate at a rate of 25 to 35 ml per min. while stirring slowly. Let stand until the pink color disappears (about 45 seconds). Heat to 55 to 60°C., and complete the titration by adding permanganate until a faint pink color persists for 30 seconds. Add the last 0.5 to 1 ml dropwise with particular care to allow each drop to become decolorized before the next is introduced.

Determine the excess of permanganate required to impart a pink color to the soln. This can be done by matching the color by adding permanganate to the same volume of the boiled and cooled diluted sulphuric acid at 55 to 60°C. This correction usually amounts to 0.03 to 0.05 ml.

EXPERIMENTAL

A solution of approximately 0.1 *N* potassium permanganate, prepared according to the A.O.A.C. method, was standardized to National Bureau of Standards, Standard Sample No. 40C, sodium oxalate, by both of the above procedures. The same solution was then standardized to arsenious oxide, and indirectly to potassium dichromate, by the following two procedures:

PROCEDURE III (5)

Aliquots of a standard soln of National Bureau of Standards Arsenious Oxide, Standard Sample No. 83, prepared according to the official A.O.A.C. procedure, (6) were diluted with 100 ml water and made ca *N* with hydrochloric acid. After the addition of 1 g of sodium chloride and 1 drop of M/400 potassium iodate soln (used as a catalyst), the mixture was titrated with the potassium permanganate to the first appearance of color. No difficulties were encountered in the titration. No perceptible lag was noted as the end point was approached, and a very sharp end point was obtained.

PROCEDURE IV (7,8)

Approximately 0.1 *N* sodium thiosulfate soln was standardized according to the A.O.A.C. procedure (9) using N.B.S. standard sample No. 136 potassium dichromate. Aliquots of the potassium permanganate soln were pipetted into a soln of 2 g of potassium iodide dissolved in 10 ml of 10% sulfuric acid. The soln was gently swirled during the addition of the permanganate soln, then diluted with 200 ml water. The released iodine was titrated immediately with the sodium thiosulfate soln, using starch as an indicator toward the end of the titration. Although this is an indirect method, it was found to be a very rapid determination and capable of giving very precise results.

Table 1 presents the results of the experiments outlined above, as well as the normality obtained for the permanganate solution by other collaborators using the N.B.S. procedure.

Although only purely volumetric methods were used in these experiments, the findings confirm those obtained by Fowler and Bright potentiometrically. The above results show that the titer obtained by the official A.O.A.C. procedure, using sodium oxalate as a primary standard, is higher than that obtained by procedures based on other primary standards. These results also show that the titer obtained by use of the N.B.S. procedure recommended by Fowler and Bright, yields normalities identical with those obtained by procedures based on other primary standards.

In addition, the results presented in the tabulation indicate that duplicate normalities can be obtained by different chemists using the N.B.S. procedure. The Associate Referee does not believe that additional collaborative determinations are necessary. No additional work on this topic is being recommended at this time.

It is recommended* that the N.B.S. procedure (Procedure II in this report) be adopted as official, first action, and be substituted for the procedure currently designated as official for the standardization of potassium permanganate solutions.

TABLE 1.—*Apparent normality of potassium permanganate solution*
(Procedures I–IV)

COLLABORATOR	I	II	III	IV
	A.O.A.C.	N.B.S.	ARSENIOUS OXIDE	POTASSIUM DICHROMATE
G. McClellan	0.1073	0.1070	0.1070	0.1071
	0.1073	0.1071	0.1070	0.1071
				0.1071
Av.	0.1074	0.1071	0.1070	0.1071
R. E. Duggan	0.1074	0.1071	0.1072	0.1071
	0.1074	0.1073	0.1070	0.1070
	0.1075	0.1071	0.1071	0.1071
	0.1074	0.1070	0.1073	0.1071
	0.1074	0.1070	0.1071	0.1070
				0.1070
Av.	0.1074	0.1071	0.1071	0.1071
E. C. Deal		0.1070		
		0.1071		
Av.		0.1071		

ACKNOWLEDGMENTS

The Associate Referee expresses the greatest appreciation for the invaluable suggestions and collaborative aid of G. McClellan and E. C. Deal of the New Orleans Station.

REFERENCES

(1) *This Journal*, **28**, 596 (1945).
(2) DUGGAN, R. E., *Ibid.*, **30**, 499 (1947).
(3) *Methods of Analysis, A.O.A.C.*, 6th Ed., **43.18**, p. 807.
(4) FOWLER and BRIGHT, R. P. 843, J. Res., N. Bur. Standards, 15 (1935).

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* For report of Subcommittee B and action by the Association, see *This Journal*, **31**, 44 (1948). The details of the method as adopted are given on page 118, *loc. cit.*

(5) Kolthoff and Furman, Vol. Anal., *II*, p. 286 (1929).
(6) *Methods of Analysis, A.O.A.C.*, **43.20**, p. 808.
(7) Kolthoff and Sandell, "Text Book of Quantitative Inorganic Analysis," p. 596. The Macmillan Co., N. Y. (1936).
(8) Hillebrand and Lundell, "Applied Inorganic Analysis," p. 153, John Wiley & Sons, N. Y. (1929).
(9) *Methods of Analysis, A.O.A.C.*, 6th Ed., **43.29**, p. 810.

REPORT ON BUFFER SOLUTIONS

By George G. Manov (National Bureau of Standards, Department of Commerce, Washington, D. C.), *Associate Referee*

There was reported last year the preparation of moderate quantities of very pure buffer salts, the directions for their use, and the *p*H values of their solutions. Announcement was made of the availability of these buffers, with the result that the first lots were exhausted much sooner than could have been anticipated.

Operations were therefore renewed in 1947 on a scale ten times that previously employed. Specifications were prepared from which bids could be obtained from commercial sources on high-purity, 250-pound lots of each of the salts. The resulting purchases were carefully sampled and analyzed for homogeneity and *p*H value. Certification of the phosphate buffer is still in progress, but it is believed that there will soon be on hand sufficient quantities of the pure buffer salts to meet all reasonable demands for several years. The solid buffers can be used to prepare solutions having *p*H values at 25°C of 4.01, 6.86, and 9.18.

With this phase of the work virtually completed, it is hoped that attention may now be turned toward collaborative work on *p*H measurements. It is planned to furnish two standard samples of pure buffer salts and two solutions whose *p*H values are unknown to the collaborator. It is hoped by this means (a) to enable the analyst to determine whether his *p*H meter is in proper operating condition and to take corrective action if necessary, and (b) obtain information concerning the accuracy with which *p*H measurements can be made by different laboratories. This information should make it possible for other groups in the Association to determine the extent to which differences in *p*H obtained by various laboratories are significant, and whether they should be made the basis of rejection of purchases or of possible court action by regulatory agencies.

It is recommended* that the study on buffer solutions be continued.

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* For report of Subcommittee A and action of the Association, see *This Journal*, 31, 44 (1948).

REPORT ON BROMIDE-BROMATE SOLUTION

By HALVER C. VAN DAME (Food and Drug Administration, Federal Security Agency, Cincinnati, Ohio), *Associate Referee*

The collaborative work on standard bromide-bromate solutions recommended in the previous report on this subject (*This Journal*, **30**, 502 (1947) has been completed.

Details of the method for the preparation and standardization of bromide-bromate solutions are published in *This Journal*, **31**, 119 (1948), under "Changes in Methods of Analysis."

Collaborators were sent a standard arsenite solution whose normality at 20°C. was 0.11259 and an unknown standard bromide-bromate solution. They were asked to standardize the bromide-bromate solution against the standard arsenite solution and also against a standard arsenite solution which they prepared. There follows the report of the collaborators. All normalities were corrected to 20°C.

COLLABORATOR	NORMALITY OF BROMIDE BROMATE SOLUTION AT 20°C.	
	USING AS_2O_3 SENT BY ASSOCIATE REFEREE	USING COLLABORATORS AS_2O_3
1	0.1086, 0.1087, 0.1088	0.1088, 0.1088, 0.1087
2	0.1087, 0.1087, 0.1087	0.1088, 0.1089, 0.1089
3	0.1086, 0.1086, 1.1086	0.1087, 0.1087
4	0.1088, 0.1087	0.1086, 0.1086
5	0.1087, 0.1087, 0.1087	0.1086, 0.1087, 0.1087
6	0.1088, 0.1088, 0.1087	0.1088, 0.1087, 0.1088
7	0.1087, 0.1087	

Average of all determinations = 0.1087

A standard bromide-bromate solution was standardized at various times over a year's period by the Associate Referee to determine its stability. The normality varied only 1 part in 1000.

CONCLUSIONS

The results on the standardizations by collaborators are in good agreement. The maximum variation from the average normality being only 2 parts in 1000 and that in only 2 of the 35 titrations made. The average variation is less than 1 part in 1000. The solution prepared as described and kept in the dark is stable and maintains its normality for a year or longer.

ACKNOWLEDGMENT

The Associate Referee wishes to thank the following members of the United States Food and Drug Administration for their cooperation as collaborators: John F. Weeks, Jr., New Orleans, Louisiana; Floyd E. Yarnell, Kansas City, Missouri; Gloria Getchell, Joyce M. Merting, and

William Horwitz, Minneapolis, Minnesota; Daniel Banes, Chicago, Illinois; James H. McGee, St. Louis, Missouri; and Sam D. Fine, Cincinnati, Ohio.

RECOMMENDATIONS*

It is recommended that the method of preparation and standardization described in this report be adopted as official, first action, and that the subject be closed.

REPORT ON STANDARDIZATION OF TITANIUM TRICHLORIDE

By Juanita E. Breit (Food and Drug Administration, Federal Security Agency, Cincinnati, Ohio), *Associate Referee*

The work of the Associate Referee was concerned with a further study on the preparation and standardization of titanium trichloride using three following methods:

(1) Standardization of titanium trichloride using Method II, *Methods of Analysis, A.O.A.C.*, 6th Ed. (1945), **21.37** (p. 290).

(2) *Ibid.*, substituting potassium dichromate for potassium permanganate.

(3) Standardization of titanium trichloride using potassium dichromate and diphenylamine indicator.

PREPARATION OF SOLUTIONS

Titanium trichloride.—A 15–16 per cent solution of technical grade titanium trichloride was thoroughly mixed and transferred to separate 50 ml. glass-stoppered flasks, which were then sealed with paraffin. This was done to insure the use of a uniform solution in the event samples were submitted for collaborative work; however, on the basis of results obtained in the standardization it was considered inadvisable to send out collaborative samples at this time. To the titanium trichloride contained in one flask (the weight of the chloride being obtained by difference) were added ca 40 ml of hydrochloric acid and the mixture diluted at 20°C to 500 ml with recently boiled distilled water. The solution was placed in a dark container with hydrogen atmosphere provision[1] and allowed to stand at least two days to permit the absorption of residual oxygen. All connections of the apparatus were sealed with lacquer.

REAGENTS

Diphenylamine indicator.—One g of diphenylamine was dissolved in 100 ml of sulfuric acid.

Potassium dichromate.—A standard soln was prepared by using about 5 g of

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* For report of Subcommittee A and action of the Association, see *This Journal*, 31, 44 (1948).

[1] *Methods of Analysis, A.O.A.C.*, 6th Ed. (1945), p. 289, Fig. 27.

U. S. Bureau of Standards potassium dichromate previously dried at 120°C. The normality was calculated at 20°C.

Potassium permanganate.—A standard solution was prepared using the official A.O.A.C. method.

PROCEDURE

1. *Methods of Analysis, A.O.A.C.*, 6th Ed. (1945), **21.37**, Method II.
2. *Ibid.*, substituting 0.1 *N* $K_2Cr_2O_7$ for 0.1 *N* $KMnO_4$.
3. Into a 500 ml flask put 50 ml of recently boiled H_2O and 25 ml of 40% (by weight) H_2SO_4. Pass a strong stream of CO_2 into the soln and add 40 ml of 0.1 *N* $K_2Cr_2O_7$. Then titrate the dichromate with $TiCl_3$ without interrupting the flow of CO_2. At the appearance of a green color add a few drops of diphenylamine indicator and continue the titration until the purple color just disappears. (In several of the titrations the indicator was added prior to the addition of $TiCl_3$. The results seemed to indicate that the diphenylamine may be added at any time)

In the results given in Table 1, the titanium trichloride was prepared by J. E. Breit using 69.1260 g (ca 50 ml) per 500 ml. It was allowed to stand 90 hours before being used for the standardization. For the results

TABLE 1.—*Results—three methods*

COLLABORATOR	METHOD	ML $KMnO_4$	ML $TiCl_3$	NORMALITY $TiCl_3$
Breit	1	40.00 (*N* 0.1038)	28.08	0.1479
Van Dame	1	40.00	28.02	0.1482
		ml $K_2Cr_2O_7$		
Breit	2	40.00 (*N* 0.1000)	26.90	0.1487
Breit	2	40.00	26.95	0.1484
Breit	3	40.00	26.55	0.1507
Breit	3	40.00	26.60	0.1504
Van Dame	2	40.00 (*N* 0.1014)	27.25	0.1489
Van Dame	2	40.00	27.28	0.1487
Van Dame	3	40.00	26.83	0.1512
Van Dame	3	40.00	26.85	0.1511

TABLE 2.—*Results—potassium dichromate*

COLLABORATOR	METHOD	ML $K_2Cr_2O_7$	ML $TiCl_3$	NORMALITY $TiCl_3$
Breit	2	40.00 (*N* 0.1000)	25.85	0.1547
Breit	2	40.00	25.83	0.1548
Breit	3	40.00	25.45	0.1572
Breit	3	40.00	25.42	0.1573
Van Dame	2	40.00 (*N* 0.1014)	26.10	0.1554
Van Dame	2	40.00	26.15	0.1551
Van Dame	3	40.00	25.84	0.1570
Van Dame	3	40.00	25.86	0.1569

obtained for Table 2, the titanium trichloride was prepared by H. C. Van Dame, using 70.3002 g per 500 ml. This solution was made 65 hours prior to use. For the determinations each analyst made and used his own 0.1 *N* potassium dichromate.

In Table 3 are given the results of a series of titrations made to determine the stability of titanium trichloride. This solution was prepared on August 4, 1947, by adding 90 ml of hydrochloric acid to ca 80 ml of titanium trichloride and diluting with water to 1200 ml. In the titrations, 50 ml of ordinary 10 per cent sulfuric acid were substituted for the 50 ml of recently boiled water and 25 ml of 40 per cent sulfuric acid.

TABLE 3.—*Results on stability*

METHOD	DATE	ML $KMnO_4$ (*N* 0.1049)	ML $TiCl_3$	NORMALITY $TiCl_3$
1	8/ 5/47	30.00	32.75	0.0961
1	8/ 5/47	30.00	32.75	0.0961
1	8/ 6/47	30.00	32.75	0.0961
1	8/ 6/47	30.00	32.78	0.0960
1	8/ 8/47	30.05	32.80	0.0961
1	8/ 8/47	30.05	32.80	0.0961
1	8/15/47	30.00	32.83	0.0959
1	8/15/47	30.00	32.85	0.0958
		ml $K_2Cr_2O_7$ (*N* 0.1036)		
2	8/ 5/47	30.00	32.32	0.0962
2	8/ 5/47	30.00	32.28	0.0963
2	8/ 6/47	30.00	32.30	0.0962
		ml $KMnO_4$ (*N* 0.1049)		
2	8/ 6/47	32.00	34.50	0.0961
2	8/ 8/47	30.00	32.30	0.0962
2	8/ 8/47	30.00	32.30	0.0962
2	8/15/47	30.00	32.40	0.0959
2	8/15/47	30.00	32.35	0.0961
2	8/29/47	30.00	32.53	0.0955
2	8/29/47	30.00	32.55	0.0955
3	8/ 5/47	30.03	31.80	0.0978
3	8/ 5/47	30.00	31.78	0.0978
3	8/ 6/47	30.02	31.82	0.0977
3	8/ 6/47	30.15	31.95	0.0978
3	8/ 8/47	30.00	31.78	0.0978
3	8/ 8/47	30.00	31.80	0.0977
3	8/15/47	30.00	31.85	0.0976
3	8/15/47	30.00	31.83	0.0975
3	8/29/47	30.00	32.00	0.0971
3	8/29/47	30.00	32.05	0.0970

On the basis of results obtained it seemed inadvisable to send out, at the present time, samples for collaborative work; however, to obtain some data on the reliability and accuracy of Method 3, four chemists of the Food and Drug Administration, Cincinnati, Ohio, standardized the same titanium trichloride solution, each using a standard potassium dichromate solution he himself had prepared. Results are given in Table 4. The titanium trichloride solution stood for 48 hours before being used and was prepared by the same method given in obtaining the results in Table 3.

TABLE 4.—*Comparable results, Method 3*

COLLABORATOR	ML N 0.1 $K_2Cr_2O_7$ (F=1.0025)	ML $TiCl_3$	NORMALITY $TiCl_3$
R. Hyatt	30.00	30.75	0.0978
R. Hyatt	30.00	30.72	0.0979
F. J. McNall	30.00	30.85	0.0975
F. J. McNall	30.00	30.90	0.0974
H. C. Van Dame	30.00	30.75	0.0978
H. C. Van Dame	30.00	30.75	0.0978
J. E. Breit	30.00	30.70	0.0980
J. E. Breit	30.00	30.70	0.0980

CONCLUSIONS

(1) That a solution of titanium trichloride is not stable.

(2) Method 3 gives a higher normality to a solution of titanium trichloride than Methods 1 and 2; however, results are consistent and can be duplicated by different chemists. Moreover, it involves the use of only two outside reagents, a primary standard, potassium permanganate, and an indicator, diphenylamine.

RECOMMENDATIONS*

It is recommended—

(1) That further study be made on the stability of titanium trichloride.

(2) That collaborative work be done on the standardization of titanium trichloride.

REPORT ON COSMETICS

By G. R. CLARK (Food and Drug Administration, Federal Security Agency, Washington, D.C.), *Referee*

The Referee makes the following recommendations:†

That the following topics on which no reports have been received be continued for the following year:

Alkalies in Cuticle Removers
Cosmetic Powders

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* For report of Subcommittee B and action of the Association, see *This Journal*, 31, 44 (1948).
† For report of Subcommittee B and action of the Association, see *This Journal*, 31, 47 (1948).

Cosmetic Skin Lotions
Deodorants and Antiperspirants
Depilatories
Hair Dyes and Rinses
Hair Straighteners
Mercury Salts in Cosmetics

The Referee concurs in the following recommendations:†

The recommendation of Charles F. Bruening, Associate Referee on cosmetic creams, that the proposed periodate and acidimetric methods be adopted as tentative for the determination of glycerol, and that the study be continued.

The recommendation of J. F. Weeks that the study of moisture in cosmetics be continued.

The recommendation of C. R. Joiner that study of the problem of pyrogallol in hair dyes be continued.

The recommendation of Paul W. Jewel that study of mascaras, eyebrow pencils, and eye shadows be continued.

REPORT ON PYROGALLOL IN HAIR DYES

By Curtis R. Joiner (Food and Drug Administration, Federal Security Agency, St. Louis, Mo.), *Associate Referee*

In a report last year (1) the Associate Referee recommended that further work be done on the extraction of pyrogallol from henna powder-pyrogallol mixtures. An outline of a method used for separating the pyrogallol from the henna was given. The final determination of the pyrogallol was made by the colorimetric method which has been adopted as tentative (2). The major difficulties encountered were incomplete recovery of pyrogallol from mixtures several months old and high blanks on henna powder.

A series of six samples were prepared and analyzed over a period of four to five months. Two samples were made from each of two different lots of henna, and for the other two, filter cel was substituted for the henna powder. Pyrogallol was mixed with filter cel in order to compare its stability when mixed with an inert material with its stability in henna mixtures. Copper sulfate and burnt sienna were added to one of the henna samples in order to prepare a mixture representative of one type of commercial preparation. Lawsone,[1] or 2-hydroxy-1, 4-naphthoquinone, the active constituent of henna for hair-dyeing purposes (3), was added to the extent of about one per cent to one of each of the henna and filter cel samples. If lawsone were the material in the henna which reacts with pyrogallol, then the addition of more lawsone should increase the rate of loss of pyrogallol from the mixture. Sixty-mesh pyrogallol was used in all samples, and all were mixed for six to eight hours.

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[1] The lawsone was synthesized from β-naphthol in four steps as given in *Organic Syntheses*, 11, 12 (1931); 21, 56 and 91 (1941); Collective Volume I, 411 (1941).

At the end of five months, analysis of two of the samples made from one lot of henna showed a loss of approximately five per cent of the pyrogallol content. One of these samples contained added lawsone, and there was no significant difference in the loss from the two. The other two henna samples showed no significant loss of pyrogallol after four months. The two filter cel samples, after five months standing, showed approximately 30 and 40 per cent losses of pyrogallol, with the sample containing the lawsone having the smaller loss. These two samples had turned noticeably gray within one week after they were prepared, and the gray color developed more slowly in the one containing the lawsone.

Analysis of a two-year old henna-pyrogallol mixture showed a loss of about 20 per cent of the original pyrogallol content.

The analyzing of all these samples at intervals will be continued, and a complete tabulation of the results will be given in next year's report.

An attempt was made to prove that some of the pyrogallol had undergone a reaction, and had not, through a physical change, become merely difficult to extract. Absorption curves of ethyl acetate extracts of a two-year old henna-pyrogallol mixture and the henna from which the mixture had been prepared were made with a Beckman spectrophotometer. The two curves were parallel and almost identical except in the range from 450 to 500 millimicrons, where there was a pronounced divergence, with the sample extract showing the greater absorption. Further work will have to be done before any conclusions can be reached.

The problem of lowering the blank is an important one since there is no simple method of evaluating it for any given sample. The variability of the blanks on different samples of henna (0.20 to 0.36 per cent) precludes the possibility of including in the method an arbitrary blank correction.

Substituting mixtures of ethyl acetate and anhydrous ether in varying proportions for the ethyl acetate used in the initial extraction lowered the blanks roughly in proportion to the percentage of ether used. The addition of 15 per cent ether by volume resulted in a 60 per cent reduction in the blank, but it also slowed up the rate of extraction of the pyrogallol to such an extent as to make it impractical to use in routine determinations, and possibly reduced the total amoung of pyrogallol recoverable. An eight-hour extraction gave 75 per cent of the recovery obtained with ethyl acetate alone.

In the method as previously outlined (1), the ethyl acetate extract was evaporated to dryness, and the residue was taken up in water and given further treatment. When the dry residue was treated with anhydrous ether, the ether filtered and evaporated to dryness and this residue taken up in water, the blanks were reduced to about 10 to 35 per cent of their former values. Blanks obtained by this method were low enough to be disregarded, but the additional treatment caused a loss of approximately 10 to 15 per cent of the pyrogallol present. Boiling the ether with the residue increased the recovery of pyrogallol only slightly.

By substituting ethyl acetate for the anhydrous ether in the treatment of the residue as given above, the blanks were reduced to 50 to 70 per cent of their former values. In a limited number of determinations, this additional treatment with ethyl acetate caused no loss of pyrogallol. The maximum blank obtained on three different henna samples was 0.20 per cent.

DISCUSSION

Dry pyrogallol, protected from light, is very stable. However, an aqueous solution in the presence of air begins to develop a yellow-brown color almost immediately. In solution pyrogallol undergoes many different types of oxidation reactions, such as: 1. To purpurogallin by sodium nitrite in acetic acid solution (4). 2. By hydrogen peroxide to purpurogallin in the presence of low concentrations of tungstic, molybdic, or vanadic acids (5). 3. In barium hydroxide solution to hexahydroxybiphenyl (6). 4. In ammoniacal silver nitrate solution to oxalic and acetic acids (7). In view of the reactive nature of pyrogallol, it is possible that, when mixed with powdered henna, it might slowly react with one or more of the constituents of the henna. Cox (3) reports that a typical specimen of henna contains, among other things: Moisture, 8.4%; sugars, as dextrose, 11.3%; gallic acid, 6.0%; hydroxynaphthoquinone (Lawsone), 1.0%. This moisture content might be sufficient to catalyze a slow oxidation reaction of added pryrogallol.

The low recoveries of pyrogallol obtained when anhydrous ether was used as a solvent might have been caused by the presence of peroxides in the ether which oxidized some of the pyrogallol. None of the ether used was tested for peroxides.

Since gallic and tannic acids both develop a blue-violet color with ferruous tartrate (8), the presence of either one in henna could account for the high blanks reported above. Alcohol, acetone, and water extracts gave very high blanks, while ether extracts were practically zero. These facts, when considered with the published solubilities of gallic acid and tannin in these solvents, indicate that tannic acid, and not gallic acid, is present in henna. However, this is contrary to the findings of Cox (3), who reported a complete absence of tannins in all samples of henna tested by him.

SUMMARY AND RECOMMENDATIONS*

Results of some investigational work indicate that pyrogallol might undergo a reaction on long standing when mixed with henna powder.

The high blank obtained from an ethyl acetate extract of henna is caused by either tannic or gallic acid in the henna. Methods involving the use of anhydrous ether as a solvent result in lower blanks, but also give low recoveries of pyrogallol. One method by which the blank is materially decreased without lowering the recovery of pyrogallol is given.

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* For report of Subcommittee B and action of the Association, see *This Journal*, 31, 47 (1948).

It is recommended that the analyses of the series of samples reported on above be continued, and that the details of the method of extraction and subsequent treatment of the extract that gave the best results be worked out and the method submitted to collaborative study.

REFERENCES

(1) Joiner, C. R., *This Journal*, **30**, 512 (1947).
(2) *Changes in Methods of Analysis*, *Ibid.*, **30**, 61 (1947).
(3) Cox, H. E., *Analyst* **63**, 397 (1938).
(4) Nierenstein, M., *Ibid.*, **70**, 213 (1945).
(5) Kar, B. C., *J. Indian Chem. Soc.*, **14**, 291 (1937). Through *C.A.*, **31**, 8341 (1937).
(6) Heinrich, F., *Sitzber. physic.-med. Sozietat Erlangen*, **71**, 199 (1939), (Pub. 1940). Through *C.A.*, **35**, 3997 (1941).
(7) Redgrove, H. S. and Foan G. A., "*Hair-Dyes and Hair-Dyeing Chemistry and Technique*" (London, 1934), p. 45.
(8) Mitchell, C. A., *Analyst*, **48**, 2 (1923).

REPORT ON COSMETIC CREAMS

DETERMINATION OF GLYCEROL IN VANISHING CREAMS

By Charles F. Bruening (Food and Drug Administration, Federal Security Agency, Baltimore, Md.), *Associate Referee*

In the Associate Referee's report of last year,[1] a collaborative study was made of the determination of glycerol in vanishing cream, using the Malaprade periodate oxidation method. Glycerol, after isolation by an extraction procedure and oxidation with potassium periodate, was determined by two methods. In the first method the amount of periodate consumed was iodometrically determined; and in the second, the amount of formic acid obtained in the oxidation was determined by titrating with alkali using methyl red indicator and applying a correction for the acidity of the potassium periodate solution used. Nine collaborators who analyzed a vanishing cream containing a known amount of glycerol by the periodate consumption method obtained an average recovery of 100% glycerol with all results falling in the range 100%±2.3%. Using the formic acid titration method and applying the correction the same collaborators obtained an average recovery of glycerol of 96.4% with a range of 96.4%±3.4%. The periodate consumption method was concluded to be satisfactory, but the formic acid titration method was considered unsatisfactory because of the low yield and difficulties encountered in determining the end points in both the formic acid titration and the acidity correction for the potassium periodate solution.

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[1] *This Journal*, **30**, 507 (1947).

A study of the formic acid method was made by Newburger and Bruening[2] and a satisfactory modified titration method for determining glycerol developed. In this method the excess of periodate, which interferes with the formic acid titration, was destroyed by using propylene glycol, and bromcresol purple indicator was substituted for methyl red. It was also shown that no correction need be made for the acidity of the potassium periodate solution. This modified method appeared promising and was submitted to collaborative study. A sample of vanishing cream of the following composition was sent to collaborators.

COMPOSITION OF VANISHING CREAM

	per cent
Stearic Acid	20.93
Water	67.83
Glycerol	10.09
Potassium Hydroxide	1.05
Propyl p-Hydroxy Benzoate	.10

The method used by the collaborators is given in detail in *This Journal*, **31**, 72 (1948).

With this vanishing cream it was suggested to the collaborators that it was convenient to use a 2 gm sample and then take a 50 ml aliquot for oxidation from the 250 ml flask. This would provide about 40 mg of glycerol. Generally, the aliquot selected should contain 30 to 40 mg of glycerol so that appropriate titrations would result in the formic acid titration method and glycerol can be determined, if desired, on the same aliquot using the periodate consumption method described in the last year's report. The suggestion was also made for this individual sample, since only formic acid titrations were to be made, that a 110 ml volumetric flask be substituted for the 100 ml flask in the oxidation, so as to permit the removal of two 50 ml aliquots for the acid titration.

As indicated in last year's report, the potassium periodate solution is subject to decomposition on standing and decreases in strength. The dry reagent grade salt is stable, and freshly prepared solutions can be used without standardizing if only the formic acid titration is desired. If, however, the periodate solution has been allowed to stand for some time, it is necessary to standardize it as described in the periodate consumption method, in order to assure that sufficient periodate is present to completely oxidize all the glycerol contained in the aliquot. Alternatively, a preliminary test may be made to assure that excess periodate remains in the oxidation mixture by adding to a test portion an excess of sodium bicarbonate and a small amount of potassium iodide. The liberation of iodine indicates excess periodate.

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[2] *Ibid.*, **30**, 651 (1947).

TABLE 1.—*Recovery of glycerol in vanishing cream*
(10.09% Glycerol)

COLLABORATOR	GLYCEROL FOUND	GLYCEROL RECOVERY
	per cent	*Average Per cent*
1	9.87 9.90	98.0
2	9.96 9.97	98.8
3	9.94 9.86	98.1
4	9.66 9.71 9.81	96.4
5	9.8 9.9	97.6
6	9.77 9.82	97.1
7	9.86 9.79	97.4
8	9.99 10.01	99.1
Average	9.87	97.8

COLLABORATORS

1. Meyer Matluck, U. S. Food and Drug Administration, Boston, Mass.
2. Sylvan H. Newburger, Cosmetic Division, U. S. Food and Drug Administration, Baltimore, Md.
3. Shirley M. Walden, U. S. Food and Drug Administration, Baltimore, Md.
4. A. T. Schramm, National Aniline Division, Allied Chemical and Dye Corporation, Buffalo, N. Y.
5. Harry Isacoff, U. S. Food and Drug Administration, New York, N. Y.
6. Edward C. Fearns, Lever Brothers Company, Cambridge, Mass.
7. Louis B. Dobie, Bristol-Myers Company, Hillside, N. J.
8. Charles F. Bruening, U. S. Food and Drug Administration, Baltimore, Md.

The results obtained by the collaborators are given in Table 1. The average recovery was 97.8% with a range of 97.8% ± 1.4%. No collaborator reported having any difficulties with the method.

When the results obtained by this method are compared with those of the formic acid method studied last year, in which the average recovery was 96.4%, with a range of 96.4% ± 3.4%, it can be readily seen that the present method is more accurate and precise. An outstanding advantage of the present method over the past one is that the color change at the end point, using bromcresol purple, is much more abrupt than the change using methyl red. In addition, when the excess of potassium periodate is removed with propylene glycol, titration difficulties caused by this compound are obviated and no acidity correction is needed for the periodate solution. It is believed that some unavoidable losses occurred in the isolation procedure which would account for a recovery somewhat less than theory. Possibly, if the initial addition of water of 25–50 ml were increased to 50–100 ml, a better recovery of glycerol may result.

ACKNOWLEDGMENT

The Associate Referee expresses his gratitude to the collaborators for their generous effort.

RECOMMENDATIONS*

It is recommended—

(1) That the periodate consumption method for the determination of glycerol in vanishing cream reported last year be made tentative.

(2) That the formic acid titration method studied in this report be made tentative.

(3) That the subject of glycerol in vanishing cream be closed and study continued on other cosmetic cream problems.

No reports were made on alkalies in cuticle removers, cosmetic powders, cosmetic skin lotions, deodorants and antiperspirants, depilatories, hair dyes and rinses, or hair straighteners.

REPORT ON MOISTURE IN COSMETICS

By JOHN F. WEEKS, JR. (Food and Drug Administration, Federal Security Agency, New Orleans, La.), *Associate Referee*

MOISTURE IN COSMETIC CREAMS

This phase of the general subject was continued. The work consisted of preparing three new cold creams of the non-mineral fat type, bearing a minimum, average, and maximum amount of water. These, together with a two-year old specimen of the same type, were subjected to drying in a forced draft oven at 100 degrees C., room pressure (about 770 mm Hg.) under varying time conditions. The samples were reduced to the consistency of soft mayonnaise (when necessary) by heating in a water bath, shaken well, about 2 g weighed out into a 7½ cm covered butter-dish, dried one-half hour, then at 15-minute intervals to constant weight.

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* For report of Subcommittee B and action of the Association, see *This Journal*, 31, 47 (1948).

Duplicate determinations showed a precision variation of 0.01–0.07%, and the accuracy varied from 0.15% to 0.26% in excess of the calculated added water.

These, together with two mineral oil type of cold creams, and three vanishing creams bearing glycerine and glycerine substitutes, were assayed by a refinement of the conventional moisture distillation method. Two size receivers, 25 ml readable to 0.05 ml, and 5 ml readable to 0.01 ml, were employed. Since no higher "reading" sensitivity was obtainable with 25 ml receiver, it was soon abandoned. The samples were mixed as before, and the soft mass sucked up into roughly calibrated tubes, immediately blown into glass-stoppered distillation flasks and weighed to the nearest milligram. A few glass beads, a small lump of rosin, and the proper amount of varsol were added and the flask connected to the remainder of the apparatus. The condenser and receiver had previously been cleaned by filling completely with cleaning solution which stood overnight in the apparatus. Distillation was allowed to proceed at the maximum safe rate, until no further increase in the water volume was noticeable in fifteen minutes heating. The flask was then immediately removed from the hot plate, and the condenser washed down with 2 ml of 0.2% Aerosol O. T. dissolved in Varsol. This operation was sufficient to sweep down completely the water entrained in the condenser. No significant flattening of the meniscus curve was observable. The trap graduate was brought to room temperature in a water bath, and read to the nearest 0.01 ml (5 ml receiver), or 0.05 ml (25 ml receiver). Flushing with water, alcohol, and ether, and drying with an induced current of air will ready it for the next determination in about five minutes.

The product, Varsol, is a standardized petroleum fraction manufactured by the Standard Oil Company. The specimen used in this work had an initial boiling point of 82 degrees C., 2% distilled at 123 degrees C., and 99% at 191 degrees C. (about 770 mm pressure).

Values on the cold creams were excellent, approaching the mathematical theory of 0.2% in the case of the 24% moisture sample, and the mineral-oil type creams gave results 0.1–0.7% of the calculated added water.

As might be expected, the accuracy of the vanishing cream assays varied by almost inverse proportion to the quantity and volatility of their glycerine or glycol constituent. It is projected that the distillation may be carried out as above, the water layer made to volume, and the glycol constituent determined by conventional methods, to give the water by difference.

It is suggested that cold creams showing less than one-half of one per cent of non-saponifiable fat be assayed for moisture by the drying method given, pending collaborative work.

It is hoped that others will have occasion to check moisture by the refined distillation method in the immediate future. Unless boil-over occurs, the original trap and condenser maybe repeatedly used without meniscus distortion. A 5 or 10 ml *modified* Bidwell & Sterling trap (Ace Glass Co.) is recommended.

Preparation and distribution of collaborative samples of nonmineral and mineral fat type cold creams is projected for the coming year. It is recommended* that the study of moisture be continued.

REPORT ON MASCARAS, EYEBROW PENCILS, AND EYE SHADOWS

By PAUL W. JEWEL (Chief Chemist, Max Factor & Co., Hollywood, Calif.), *Associate Referee*

ANALYSIS OF MASCARA

Additional surveys have been made of various brands of mascara offered for sale, and this survey indicates that more and more brands are shifting from the conventional formulation, using triethanolamine soaps, to that using potassium or sodium soaps. This makes it impossible to devise a general method which will be applicable to all types.

The method previously described[1] is satisfactory for the type which is based on mineral soaps. However, a new method developed during the past year must be used for those which contain triethanolamine soaps. This method will be described in detail in the experimental portion of this report.

ANALYSIS OF EYEBROW PENCILS

Methods for the analysis of products of this type are still far from complete. The same technique used for mascaras containing triethanolamine soaps may be used but the extraction time must be prolonged by some five or six hours.

ANALYSIS OF EYE SHADOWS

A tentative method is offered for this type of preparation for the first time. Eye shadows are very simple preparations which give little difficulty. They consist of mixtures of oils and waxes into which is milled sufficient pigments of the proper shade. The base is mostly mineral oil with small amounts of paraffin or ceresin wax, and possibly small amounts of lanolin. The pigments are usually titanium dioxide, ultramarine blue, iron oxide browns, carbon black, and in some cases chromium oxide green.

EXPERIMENTAL METHODS

MASCARA

Place a small piece of mascara in a beaker, add 10 ml chloroform, and boil for a few minutes. If the mass disintegrates and appears to dissolve completely except for the pigments, it may be safely assumed that this is one in which the soaps are based on triethanolamine. If the mass dissolves slightly or not at all, the reverse is probably true.

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* For report of Subcommittee B and action of the Association, see *This Journal*, **31**, 48 (1948).

[1] *This Journal*, **29**, 32 (1946).

If the soaps present are formed from mineral alkalis, proceed with the method previously described.[1]

If the soaps are formed from triethanolamine proceed according to the following method:

Total Base.—Weigh about three grams of mascara, wrap tightly in Munktell 00 filter paper, turning the ends under and tying with a thread to hold the paper in place. Place the wrapped sample in an extraction thimble, and extract in a Soxhlet extractor for eight hours with chloroform. Tare the extraction flask and wrap the extraction chamber with a towel so as to keep the sovent in the extraction chamber, as nearly as possible, at the boiling point. When extraction is complete, evaporate the solvent and weigh. The weight of the extract will be the total base in the mascara.

Triethanolamine.—To the base contained in the extraction flask add 25 ml of 0.50 *N* sulfuric acid, heat until the waxes melt, and allow to cool with occasional shaking. As soon as the mixture has cooled to room temperature, filter into a titration flask washing the residue and filter well with distilled water. Do not attempt to remove the solid waxes from the flask. Titrate the excess acid in the filtrate with 0.50 *N* sodium hydroxide using methyl red as an indicator. Each ml of 0.50 *N* acid used is equivalent to 0.07143 grams of triethanolamine.

Acid Number of Base.—After removal of the triethanolamine transfer any of the base which has come over onto the filter back to the flask. Pour several small portions of boiling absolute alcohol through the filter into the flask. The total should not exceed 25 ml. Heat to dissolve the waxes and titrate with 0.50 *N* alcoholic potassium hydroxide using phenolphthalein as an indicator. Multiply the ml of normal alkali used by 56.1 and divide by the sample weight to determine the acid number.

Arsenic and Lead.—The method previously described[1] is adequate. The digestion may be done on the extracted pigments if desired, or a sample, one–two grams, of the original material may be used.

EYEBROW PENCILS

Total Base.—Proceed as described under Mascara except that the extraction should be continued for 16 hours.

Arsenic and Lead.—Proceed as directed under Mascara.

EYE SHADOWS

Total Base—Weigh a sample, ca 5.0000 grams, and place in a 250 ml beaker. Add 50 ml petroleum ether and heat to gentle boiling for five minutes. Allow the pigments to settle and decant through a dry filter into a tared dish. Evaporate the solvent and weigh. Repeat the extraction until no further increase in weight is noted, finally bringing all of the pigments onto the filter.

Arsenic and Lead.—Place the filter paper with the extracted pigments in a Kjeldahl flask and proceed as directed under Mascara.

RECOMMENDATIONS*

It is recommended that work be continued on this project and that samples be submitted for collaborative study as soon as it appears that the methods are sufficiently workable to make such a study feasible.

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* For report of Subcommittee B, and action of the Association, see *This Journal*, 31, 48 (1948).

No report was made on mercury salts in cosmetics.

REPORT ON COAL-TAR COLORS

By G. R. Clark (Food and Drug Administration, Federal Security Agency, Washington, D.C.), *Referee*

The Referee makes the following recommendations:*

That the proposed method for the determination of aniline in coal-tar colors, recommended as tentative by the Associate Referee, be adopted as official, first action, and that the study of volatile amine intermediates be continued.

The Referee concurs in the following recommendations:

The recommendation of K. A. Freeman that the proposed titanium trichloride titration method be adopted as tentative for the determination of pure dye in D&C Red No. 7 and D&C Red No. 10 and lakes of these colors, and that study of lakes and pigments be continued.

The recommendation of K. A. Freeman that studies of the following topics be continued: Identification of certified coal-tar dyes, non-volatile unsulfonated amine intermediates in coal-tar colors, sulfonated amine intermediates in coal-tar colors, sulfonated phenolic intermediates, intermediates derived from phthalic acid, and halogens in halogenated fluoresceins.

The recommendation of W. C. Bainbridge that the study of mixtures of coal-tar colors for drug and cosmetic use be continued.

The recommendation of Mr. L. Koch that study of subsidiary dyes in D&C colors be continued.

The Referee further recommends:

That the following topics be reassigned to Associate Referees as listed:

Halogens in Halogenated Fluoresceins.—Nathan Gordon, Food and Drug Administration, Washington, D. C.

Nonvolatile Unsulfonated Amine Intermediates in Coal-tar Colors.—L. S. Harrow, Food and Drug Administration, Washington, D. C.

Sulfonated Amine Intermediates.—Nathan Ettelstein, Food and Drug Administration, Washington, D. C.

Intermediates Derived from Phthalic Acid.—Charles Graichen, Food and Drug Administration, Washington, D. C.

Lead in Coal-tar Colors. Nathan Ettelstein, Food and Drug Administration, Washington, D. C.

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* For report of Subcommittee B and action of the Association, see *This Journal*, 31, 48 (1948).

That an attempt be made to obtain another Associate Referee to study sulfonated phenolic intermediates.

That the following new topics be added and the Associate Referees appointed as follows:

Lead in Coal-Tar Colors.—Nathan Ettelstein, Food and Drug Administration, Washington, D. C.

Lead in Lakes of Coal-tar Colors.—L. S. Harrow, Food and Drug Administration, Washington, D. C.

Subsidiary Dyes in FD&C Colors.—M. Dolinsky, Food and Drug Administration, Washington, D. C.

Hygroscopic Properties of Coal-tar Colors.—Charles Stein, Food and Drug Administration, Washington, D. C.

That study be continued on topics for which no report was received, as follows:

Acetates, carbonates, halides, and sulfates in certified coal-tar colors.
Buffers and solvents in titanium trichloride titrations.
Ether extract in coal-tar colors.
Unsulfonated phenolic intermediates in coal-tar colors.

No report was given on acetates carbonates, halides, and sulfates in certified coal-tar colors or on buffers and solvents in titanium chloride titrations.

REPORT ON ETHER EXTRACT IN COAL-TAR COLORS

By S. S. Forrest (Cosmetic Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

Since little work has been done on ether extracts from fluorescein colors, it was decided to investigate this problem. D&C Orange No. 5 was chosen for this study since it is one of the fluorescein colors most frequently submitted for certification.

A method which appeared to give good results was submitted to collaborative study. Samples with the method were sent to various collaborators of whom four replied in time for this report. Listed in the order received, they were:

Ansbacher-Siegle Corporation, H. Holtzman reporting.
H. Kohnstamm and Company, Inc., Samuel Zuckerman reporting.
Bates Chemical Company, Inc., C. O. Beecher reporting.
Wm. J. Stange Company, W. H. Kretlow reporting.

METHOD

Dissolve 5 g of the color in 200 ml of 2% NaOH, place in a continuous extractor and extract with 100 ml of ethyl ether for 3–4 hours. Transfer the extract to a separatory funnel, wash with successive 10 ml portions of ca 0.1 *N* NaOH until the washings are colorless, then with 10 ml portions of distilled water until the washings are neutral to litmus paper. Decant the ether into a weighed evaporatng dish, allow to evaporate at about 40°C., dry over calcium chloride, and weigh. The increase in weight represents "ether extract."

As may be seen from the table, the results are widely divergent. It appears therefore that the method should be studied further.

TABLE 1.—*Collaborative results: ether extracts from D&C Orange No. 5*

COLLABORATOR	
1	0.19 0.15
2	0.050 0.048 0.040
3	0.08 0.108 0.036
4	0.13 0.10
Associate Referee	0.040 0.048

RECOMMENDATION*

It is recommended that the topic be continued.

No report was given on identification of certified coal-tar colors.

REPORT ON HALOGENS IN HALOGENATED FLUORESCEINS

By N. GORDON (Cosmetic Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

IODINE

A rapid method for the determination of iodine in soluble iodides and in organic compounds has been reported by Clark and Jones.[1] The method involves oxidation of the iodine to iodate in an acid-permanganate medium, removal of excess permanganate and manganese dioxide, addition of potassium iodide, and titration of the liberated iodine with standard thiosulfate solution.

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* For report of Subcommittee B and action of the Association, see *This Journal*, 31, 48 (1948).

[1] *This Journal*, 25, 755 (1942).

A collaborative study of this method has been undertaken. Samples of FD&C Red No. 3 and D&C Red No. 3, Aluminum Lake, were submitted with the method to the following collaborators listed alphabetically:

Bates Chemical Company, Inc., C. O. Beecher reporting.
Max Factor, Inc., P. W. Jewel reporting.
Hilton-Davis Chemical Company, Anna Bartruff reporting.
H. Kohnstamm and Company, Inc., I. Hanig reporting.
National Aniline Division, Allied Chemical and Dye Corporation, A. T. Schramm reporting.
Wm. J. Stange Company, W. H. Kretlow reporting.
Cosmetic Division, Food and Drug Administration, L. S. Harrow and C. Stein reporting.

In some cases, two or more reports were received from the same laboratory, making a total of twelve reports. Six collaborators reported no difficulty with the method, while the others experienced various degrees of difficulty. The principal recommendations and comments were:

1. The addition of the dilute potassium permanganate solution dropwise, instead of in 1 ml portions to reduce incidence of brown color formation.

Analyses were conducted following this recommendation. No difference in results were found.

2. Difficulty in obtaining the permanent pink after filtration, and the appearance of a brown coloration during filtration, even after repeated treatment with additional $KMnO_4$.

These difficulties appear to be due to the addition of excess potassium nitrite solution.

3. Difficulty in ridding the solution of manganese dioxide and in filtering.

The method emphasizes that no attempt should be made to destroy the remaining particles of manganese dioxide. Filtration is used to remove these particles of manganese dioxide from the solution.

4. More explicit description of the proper addition of potassium nitrite was requested by two collaborators. This step is the only truly critical point in the determination.

A revised procedure is being prepared to include a more detailed description of this step.

5. After the addition of sulfamic acid, the statement "swirl the contents until the evolution of gas ceases" has been pointed out to be meaningless, as only a very small volume of gas will be produced.

Accordingly, the revised procedure will not contain this statement.

All results, listed in the order they were received, are shown in Table 1. The results of the collaborators and of the Associate Referee are in fair agreement.

CHLORINE

A method for the determination of chlorine and bromine in the presence of each other has been reported by Clark and Jones.[2]

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[2] *This Journal*, 26, 316 (1943).

The chloride and bromide ions are determined in the presence of each other by precipitating the combined silver halides from one protion of the sample and determining the bromide in another portion. Thus bromine is determined directly and chlorine by difference.

The bromine determination depends upon oxidation with an excess of permanganate in a phosphoric acid solution containing cyanide. Under these conditions the bromide is quantitatively converted to bromine cyanide while the chloride is not oxidized. After the excess permanganate is reduced with ferrous ammonium sulfate, potassium iodide is added, and the liberated iodine titrated with thiosulfate.

TABLE 1.—*Collaborative results of iodine in halogenated fluoresceins*

COLLABORATOR	FD&C RED NO. 3 FOUND	D&C RED NO. 3, AL. LAKE FOUND
	per cent	*per cent*
Associate Referee	50.0	9.3
2	49.5	9.3
3	50.5	9.3
4	49.6	9.3
5	49.0	9.1
6	50.0	9.4
7	49.6	9.6
8	48.5	9.0
9	50.9	9.2
10	50.1	9.4
11	49.3	—
12	—	9.2
Average per cent	49.7	9.3
Average deviation	±0.5	±0.1

The method for determining bromine is official, first action, A.O.A.C. **21.53,** Sixth Edition.

Samples of D&C Red No. 27 and directions for analysis were submitted to the following collaborators:

Max Factor, P. W. Jewel reporting.

H. Kohnstamm and Company, Inc., R. C. Cooney and Virginia Schmuckle reporting.

Wm. J. Stange Company, W. H. Kretlow reporting.

Cosmetic Division, Food and Drug Administration, R. N. Sclar reporting.

The sample submitted was a commercial product. In some cases, two reports were received from the same laboratory.

A total of six reports containing analytical results have been received.

One collaborator reported that his results for bromine were too variable to warrant continuation of the analysis for chlorine.

Another collaborator experienced difficulty duplicating results when separate gravimetric samples were employed. However, he reported good checks upon using aliquot portions of a 1% solution.

All results, listed in the order they were received, are shown in Table 2.

TABLE 2.—*Collaborative results for chlorine and bromine in halogenated fluoresceins*

COLLABORATOR	D&C RED NO. 27	
	CHLORINE	BROMINE
	Per cent	*Per cent*
Associate Referee	17.0	37.8
2	20.3	37.6
3	—	37.2
4	15.8	37.7
5	17.6	37.5
6	—	—

The results of the collaborators and of the Associate Referee for bromine are in fair agreement. The results for chlorine are poor and in disagreement.

RECOMMENDATIONS*

It is recommended that—

(1) The iodine method be revised to include a more detailed explanation of the potassium nitrite addition.

(2) The revised method be submitted to collaborators for investigation.

(3) More work be done on the chlorine method.

VOLATILE AMINE INTERMEDIATES IN COAL-TAR COLORS

By ALICE B. CAEMMERER (Cosmetic Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

The methods most commonly used for the quantitative determination of free volatile amine intermediates in coal-tar colors involve colorimetric procedures. However, visual colorimetric determinations are subject to personal errors because of the variation in visual perception.

The method proposed involves separation of the intermediate by steam distillation, followed by diazotization, and coupling with an appropriate phenolic derivative. The resulting dye is titrated with standard titanium trichloride solution. The weight of amine intermediate may then be readily computed. The equipment used is normally found in all color laboratories, and is inexpensive and readily replaced. Details of the method are given in *This Journal,* **31,** 81 (1948), under "Changes in Methods of Analysis."

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* For report of Subcommittee B and action of the Association, see *This Journal*, 31, 48 (1948).

In experiments conducted by the Associate Referee, recoveries of known amounts of aniline averaged 97 per cent. In these experiments, the method was applied to recoveries of added aniline from D&C Black No. 1, D&C Red No. 17, D&C Red No. 31, D&C Red No. 33, and Ext. D&C Yellow No. 3, Lake. Other members of the Color Certification Section also obtained good results in trials of the proposed method.

COLLABORATIVE RESULTS

Samples of D&C Orange No. 3 and FD&C Yellow No. 3 containing known amounts of aniline were prepared and distributed to collaborators. The results obtained are shown in Table 1.

TABLE 1.—*Collaborative results*

	FD&C YELLOW NO. 3	D&C ORANGE NO. 3
	per cent	*per cent*
Aniline added	0.20	0.20
Collaborator No. 1	0.090	0.086
	0.086	0.074
Collaborator No. 2	0.19	0.20
	0.19	0.19
Collaborator No. 3	0.1916	0.1916
	0.1930	0.1890
Collaborator No. 4	0.20	0.30
	0.20	
Associate Referee	0.20	0.20
	0.20	0.20

With one exception, the collaborators obtained results agreeing very closely with the actual amount of aniline present.

The Associate Referee wishes to acknowledge the assistance of the following collaborators:

Ansbacher-Siegle Corporation, H. Holtzman reporting.
Harmon Color Works, Inc., V. C. Vesce (per J. W. Ingram) reporting.
H. Kohnstamm and Company, Inc., L. Koch reporting.
Wm. J. Stange Company, W. H. Kretlow reporting.

FURTHER STUDIES

Since the method was submitted to collaborative study, investigations have been made to determine its applicability to other aromatic amine intermediates that are volatile with steam. Good recoveries have been obtained for p-toluidine by the Associate Referee.

RECOMMENDATIONS*

It is recommended—

(1) That the method for volatile amine intermediates be adopted as tentative for aniline.

(2) That the method be further studied to determine its applicability to volatile amine intermediates other than aniline.

NON-VOLATILE UNSULFONATED AMINE INTERMEDIATES IN COAL-TAR COLORS

By Lee S. Harrow (Cosmetic Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

D&C Black No. 1 (also known as Napthol Blue Black, C. I. 246) is one of the colors certifiable by the Food and Drug Administration for coloring drugs and cosmetics.[1] The free intermediate, p-nitroaniline, is permitted in a certified batch of this color to the extent of not more than 0.2 per cent.

The method proposed herein consists of extracting the p-nitroaniline from the color with ether and titrating the nitro group with titanium trichloride.[2]

METHOD

DETERMINATION

Place a 10 g sample of D&C Black No. 1 in a Soxhlet extraction thimble. Extract with ether until the flushings are colorless. (This should take at least three hours.) Transfer the extract to a 500 ml wide-mouth Erlenmeyer flask. Rinse the flask with two 10 ml portions of ether and add these to the main extract. Add 50 ml of water to the extracts and evaporate on a steam bath until all the ether is driven off, using a gentle air blast to hasten the process. Remove the flask from the steam bath, cool to room temp, and add ca 15 g of sodium tartrate. Heat the soln to boiling and titrate with standard 0.1 *N* titanium trichloride under a stream of carbon dioxide to the disappearance of the yellow color. The end point can be more readily detected when 1 ml of a standard soln of FD&C Green No. 2 (Light Green SF Yellowish) is added near the end of the titration to serve as an indicator.

RESULTS

A sample of D&C Black No. 1 was purified by repeated extraction with ether until no more intermediate was shown by this method. Known quantities of p-nitroaniline were added to 10 gm. portions of the color and tested for recovery. Results obtained with the method are shown in Table 1.

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* For report of Subcommittee B and action of the Association, see *This Journal*, 31, 48 (1948).

[1] Food and Drug Administration, S.R.A., F.D.C. 3 (1940).

[2] Knecht and Hibbert, "New Reduction Methods in Volumetric Analysis," Longmans, 1918.

TABLE 1.—*Recovery of p-nitroaniline from D&C Black No. 1*

p-NITROANILINE ADDED	p-NITROANILINE ADDED	p-NITROANILINE RECOVERED	RECOVERY
per cent	*gram*	*gram*	*per cent*
	0.00	0.00	
0.2	0.0199	0.0189	94.8
	0.0200	0.0190	95.0
	0.0200	0.0195	97.1
0.4	0.0400	0.0378	94.4
	0.0403	0.0399	99.0
	0.0404	0.0404	100.0
0.6	0.0606	0.0603	99.6
	0.0600	0.0593	98.8
	0.0610	0.0608	99.7

Further investigations are being conducted to determine the applicability of the method to 2-4-dinitroaniline, 2-nitro-p-anisidine, and 3-nitro-p-toluidine in colors in which they may be encountered.

SUMMARY

A titrametric method has been presented for the quantitative determination of p-nitroaniline in D&C Black No. 1. The intermediate is removed from the color by ether extraction and quantitatively estimated by titration with titanium trichloride. Recoveries of over 95 per cent have been obtained when the method was used to determine 0.2 to 0.6 per cent of the intermediate.

No reports were given for sulfonated phenolic intermediates, intermediates derived from phthalic acid, or mixtures of coal-tar colors for drug and cosmetic use.

PURE DYE IN LAKES AND PIGMENTS

By KENNETH A. FREEMAN (Cosmetic Division, Food and
Drug Administration, Federal Security Agency,
Washington, D. C.), *Associate Referee*

The A.O.A.C. has adopted as tentative a titration procedure for D&C Red No. 31 and D&C Red No. 8. (1) At the annual meeting in October 1946 a report was presented further showing the accuracy of the method. (2) Since that time pure D&C Red No. 7 has been prepared, analyzed by various methods, and the results compared with the titration method. The results agreed within ±0.8 per cent.

It appeared, therefore, that the titration procedure could be applied to D&C Red No. 7. Clark (3) has shown the method to be applicable to D&C Red No. 10.

Accordingly, samples of D&C Red No. 7, Calcium Lake, D&C Red No. 8, Sodium Lake, D&C Red No. 10, Sodium Lake, and D&C Red No. 31, Calcium Lake, were submitted to various laboratories for collaborative study. Samples with directions were sent to the following, listed alphabetically:

Ansbacher-Siegle Corporation, H. Holtzman reporting.

Calco Chemical Division, American Cyanamid Company, Mrs. E. Z. Montgomery reporting.

Harmon Color Works, Inc., Vincent C. Vesce reporting (per J. W. Ingram).

Hilton-Davis Chemical Company, Anna Bartruff reporting.

H. Kohnstamm & Company, Inc., Eugene F. Wojt reporting.

National Aniline Division, Allied Chemical & Dye Corporation, A. T. Schramm reporting.

Wm. J. Stange Company, W. H. Kretlow reporting.

U. S. Food & Drug Administration, Cosmetic Division, Lee S. Harrow, S. S. Forrest, and Charles Stein reporting.

The results are listed in Table 1 in the order in which they were received.

An inspection of the table discloses that the results of each collaborator are consistent. In cases where the collaborators results are high for one dye, they are generally high for all four dyes. Where they are low for one they are generally low for all four. It is the opinion of the Referee that this is due to variation in the standard titanium trichloride solutions used rather than to failure of the method. The A.O.A.C. Book of Methods describes two tentative methods for standardizing titanium trichloride solutions. Method I uses Bureau of Standards ingot iron as a primary standard while Method II uses standard potassium permanganate solution. Our experience in the color certification laboratory has shown that Method I gives unsatisfactory results because the Bureau of Standards iron is not pure, nor is the purity known. The Bureau of Standards itself points out this fact. If, therefore, the collaborators standardized their titanium trichloride solutions with iron, the results would vary with the purity of the iron used for standardization. Titanium trichloride is standardized by the color certification laboratory by Method II except that Bureau of Standards potassium dichromate is used instead of potassium permanganate. This has been found to be more convenient and equally accurate, and should have been specified in the submitted method.

The collaborators encountered some difficulty with the method for all colors except D&C Red No. 7. D&C Red No. 10 appeared to give the most trouble since three collaborators each reported some difficulty.

Their comments were:

"End point of titration was variable in the shade of the reduction product."

" . . . We found the titration to be sensitive to small differences in acidity, a

TABLE 1.—*Collaborative results*

COLOR	COLLABORATOR	PURE DYE	AVERAGE VALUE	STANDARD DEVIATION
		per cent	*per cent*	*per cent*
D&C Red No. 7 Calcium Lake	1	24.7		
	2	26.6		
	3	24.4		
	4	26.6		
	5	25.7		
	6	25.8		
	7	25.9		
	8	25.5		
	9	25.6		
	Referee	25.5	25.6	±0.7
D&C Red No. 8 Sodium Lake	1	73.8		
	2	73.8		
	3	73.2		
	4	76.7		
	5	74.4		
	6	75.8		
	7	75.9		
	8	73.2		
	9	73.2		
	Referee	73.6	74.4	±1.1
D&C Red No. 10 Sodium Lake	1	92.4		
	2	92.3		
	3	90.0		
	4	91.7		
	5	92.0		
	6	92.2		
	7	92.2		
	8	90.9		
	9	92.3		
	Referee	91.2	91.7	±0.7
D&C Red No. 31 Calcium Lake	1	*		
	2	44.8		
	3	44.3		
	4	47.5		
	5	45.4		
	6	45.2		
	7	43.6		
	8	42.9		
	9	45.3		
	Referee	44.2	44.9	±1.2

* This collaborator reported inability to obtain a satisfactory end point.

slightly more alkaline condition producing a cloudy soln with an orange, instead of a yellow end point. In such conditions low results were obtained, whereas under the proper condition there was no difficulty in obtaining a clear soln, yellow end point, and good duplicability."

"It was observed that the D&C Red No. 10 decomposed when allowed to stand in contact with conc. H_2SO_4 for a prolonged period of time."

In the case of D&C Red No. 8 two collaborators reported a slow fading of the end point but were able to obtain reasonably good checks.

D&C Red No. 31 also gave some trouble, one collaborator observing that duplicability was poorest with this color. Another reported "Sodium bitartrate precipitated the dye, making the titration impossible. Alcohol did not remedy the situation."

One collaborator suggested a back titration procedure to avoid uncertainty as to the true end point, particularly in those cases where the end point is a gradual change from orange to yellow. This suggestion was studied by the Associate Referee and found to be sound. Reserve samples of each color submitted to collaborative study were analyzed by the back titration procedure. The results were the same but in some cases the end points were easier to see. Therefore, the method is revised to include the back titration procedure at the option of the analyst.

RECOMMENDATIONS*

It is recommended that—

(1) The method be adopted as tentative for D&C Red No.'s 7 and 10 and their lakes.

(2) The following be inserted; "If the end point is indistinct, overtitrate by ca 0.5 ml of the titanium trichloride solution and back-titrate with standard indicator solution substracting the indicator blank from the standard titanium trichloride solution used."

(3) The study of Lakes and Pigments be continued.

REFERENCES

(1) *Methods of Analysis, A.O.A.C.*, 6th Ed., **21.41(d)**, p. 291.
(2) Freeman, Kenneth A., *This Journal*, **30**, 520 (1947).
(3) Clark, G. R., *Ibid.*, **28**, 761 (1945).

REPORT ON SPECTROPHOTOMETRIC TESTING

ANALYSIS OF COAL-TAR COLORS
EXT. D&C ORANGE NO. 1

By Rachel N. Sclar (Cosmetic Division, Food and
Drug Administration, Federal Security Agency,
Washington, D. C.), *Associate Referee*

Spectrophotometric analysis of oil-soluble dyes certifiable under the

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* For report of Subcommittee B and action of the Association, see *This Journal*, **31**, 48 (1948).

Coal-Tar Color Regulations[1] is extended in this report to Ext. D&C Orange No. 1 (Hansa Orange). Previous reports gave spectrophotometric data for Ext. D&C Yellow No. 5[2] and for D&C Red No. 35[3] and D&C Red No. 36.[3]

Agreement with Beer's law, location of the absorption peak, and the extinction ratio were determined for solutions of this dye.

Data were obtained with a General Electric recording spectrophotometer equipped with an automatic slit adjustment for an 8 millimicron wave-length band.

EXPERIMENTAL

Preparation of Standard Dyestuff.—3 nitro-4-aminoanisole (m.p. 123°) was diazotized and coupled with aceto-aceto-ortho-toluidide (m.p. 103°) in alkaline solution. The product was washed thoroughly with hot water, dried and recrystallized from benzene; it melted at 210°C (on Fisher Block). A portion of this product recrystallized from chloroform showed the same melting point.

TABLE 1.—*Extinction values of solutions of Ext. D&C Orange No. 1 in U.S.P. chloroform*

Typical Data

CURVE NO. (CHART 1)	CONCENTRATION	EXTINCTION			E_{432} mμ / CONCENTRATION	E_{420} mμ / E_{450} mμ
		420 mμ	432 mμ	450 mμ		
	mg./liter					
1	3.17	.150	.158	.136	.0498	1.103
2	6.33	.300	.316	.272	.0499	1.103
3	12.66	.598	.632	.544	.0499	1.099
4	25.33	1.196	1.264	1.086	.0499	1.101
					Av. .0499	Av. 1.10

A sample of the dye, made commercially but with a special effort to obtain purity, melted at 207.5°C. Spectrophotometric examination of this material showed the extinction per milligram/liter to be lower than that of the material prepared in this laboratory. When the commercial sample was washed with hot water and recrystallized first from benzene, then from chloroform, the melting point and extinction per milligram/liter were found to agree with those of the laboratory sample. The laboratory sample was therefore considered sufficiently pure to serve as a standard.

Preparation of Solutions.—A 25.33 mg portion of dye weighed on a

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[1] U. S. Food and Drug Administration, Service and Regulatory Announcements, F.D.C. 3.

[2] G. R. Clark and S. H. Newberger, *This Journal*, 27, 576 (1944).

[3] Rachel N. Sclar, *Ibid.*, 30, 522 (1947).

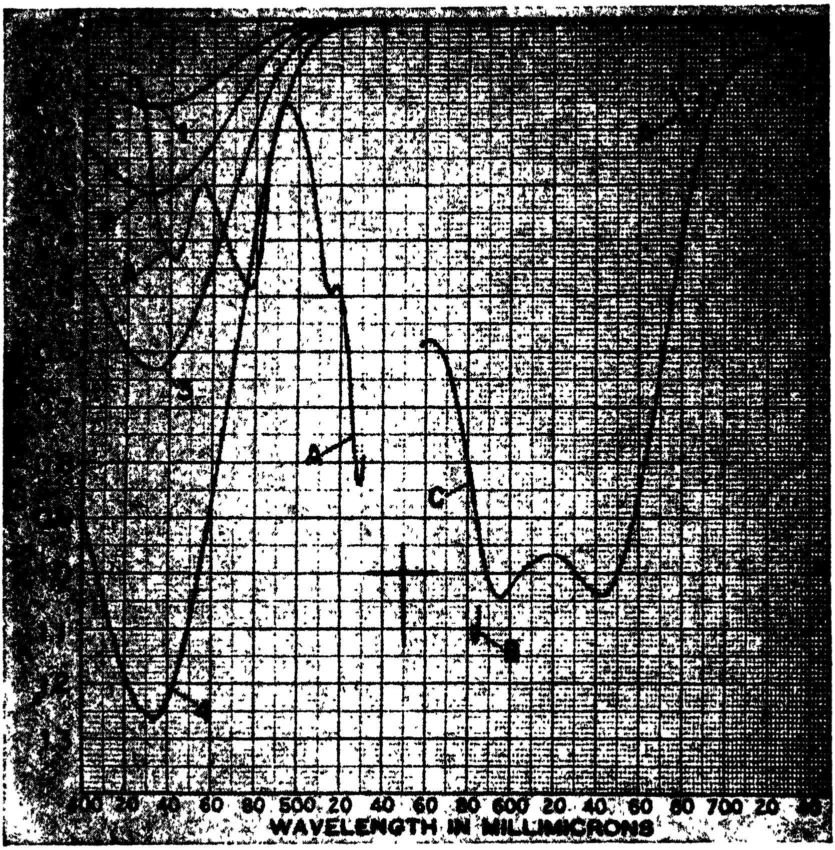

CHART 1.—Pure Ext. D&C Orange No. 1 in chloroform.

Curve 1— 3.17 mg./liter.
Curve 2— 6.33 mg./liter.
Curve 3—12.66 mg./liter.
Curve 4—25.33 mg./liter.
Cells—1 cm.
A—Corning Didymium Glass 512, 6.0 mm.
(Absorption peaks at 400.4, 441.4, 477.1, 529.0, and 684.8 mμ.)
B—Corning Didymium Glass 592, 4.02 mm.
(Absorption peak at 583.7 mμ.)
C—Signal Lunar White Glass H-6946236.

semimicro balance sensitive to 0.02 mg, was dissolved in about 50 ml of chloroform in a 100 ml volumetric flask. The solution was made to volume with chloroform and aliquot portions diluted with chloroform to the concentrations shown in Table 1. All solutions were made to volume at the temperature of the room in which the optical measurements were made. U.S.P. chloroform was used throughout the experiment.

In subsequent experiments, it was found that gentle warming on a

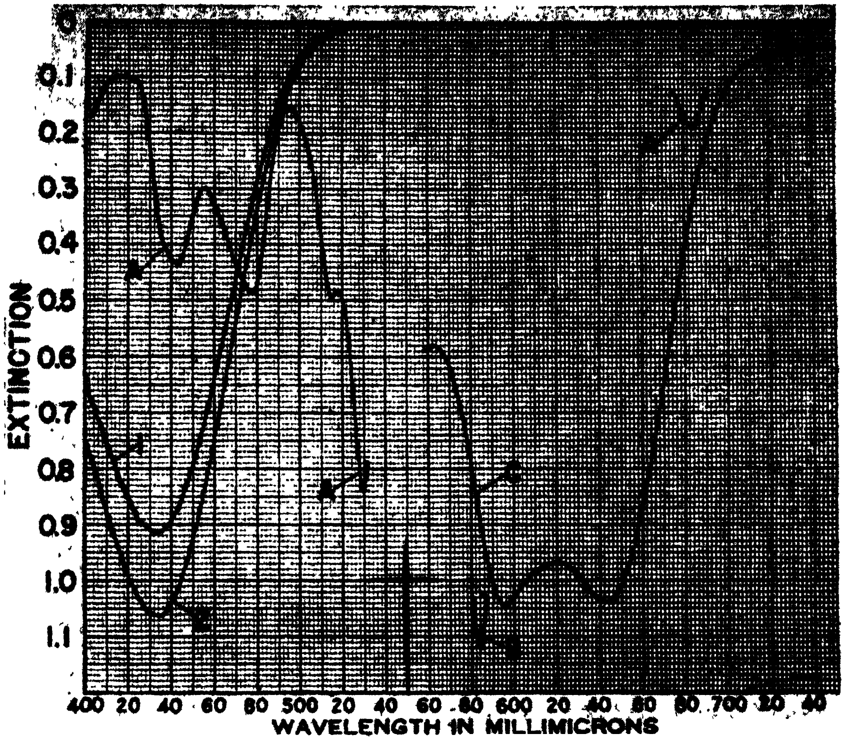

Chart 2.—Certified samples of Ext. D&C Orange No. 1.

Curve 1—Primary Color—18.63 mg./liter.
Curve 2—Lake —42.09 mg./liter.
Cells—1 cm.
A—Corning Didymium Glass 512, 6.0 mm.
(Absorption peaks at 400.4, 441.4, 477.1, 529.0, and 684.8 mμ.)
B—Corning Didymium Glass 592, 4.02 mm.
(Absorption peak at 583.7 mμ.)
C—Signal Lunar White Glass H—6946236.

water bath facilitated the solution of samples of the color. No adverse effects from this treatment were noted.

The stability of the color in chloroform solution was investigated by aging a diluted aliquot of a master solution for three days in the dark. When this solution was made to volume spectrophotometric examination showed no fading of color when compared with a diluted aliquot examined at the beginning of this period.

Spectrophotometric Data.—The extinction curves for the chloroform solutions of Ext. D&C Orange No. 1 are shown in Chart 1. They show an absorption peak at 432±2 mμ. (All wave lengths were corrected to ±2 mμ with the aid of didymium glasses tested by the National Bureau of

Standards; see footnote to Charts 1 and 2.) Average extinction per milligram/liter at this wave length was found to be .0499.

The ratio of extinction values at 420 mμ and 450 mμ was calculated This ratio E_{420} mμ/E_{450} mμ = 1.10 ± .005; see Table 1.

DISCUSSION

The ratios of extinction to concentration in Table 1 indicate that at 432 mμ, chloroform solutions of Ext. D&C Orange No. 1 containing 3.2 to 25.3 mg of color per liter obey Beer's law. The pure dye content of a sample of this color can therefore be determined from spectrophotometric data of its solution by comparison with the experimentally determined standard.

APPLICATION TO COMMERCIAL SAMPLES

Primary Colors and Lakes

One sample of certified Ext. D&C Orange No. 1 and one sample of Ext. D&C Orange No. 1, Lake (made by extending the dye on insoluble substrata) were analyzed spectrophotometrically. Weighed samples were warmed on the steam bath with U.S.P. chloroform to dissolve the color. It was necessary to filter the solution obtained from the lake through a fine sintered glass crucible to remove the substratum. The solutions were transferred to 100 ml flasks and made to volume at room temperature. Extinction measurements were made on suitably diluted aliquots. The curves are shown in Chart 2, and the data in Table 2.

TABLE 2.—*Analysis of certified samples of Ext. D&C Orange No. 1: a primary color and a lake*

SAMPLE NO.	M.P.	CONCENTRATION OF SAMPLE	E_{432} mμ	DYE[1] SPECTROPHOTOMETRICALLY	DYE FROM CHEMICAL ANALYSIS
	°C.	*mg /liter*		*per cent*	*per cent*
Primary Color	209	18.63	.916	98.5	98.5 (from N_2 content)
Lake[2]		42.09	1.064	50.7	51 0 (by extraction)[3]

[1] The dye content was calculated by using .0499 (Table 1) as the extinction value for 1 mg./liter of Ext. D&C Orange No. 1.

[2] Substratum—alumina and talc.

[3] The color was extracted from the lake with benzene, the solvent evaporated, and the extracted material weighed.

SUMMARY

Spectrophotometric data for chloroform solutions of purified Ext. D&C Orange No. 1 are presented. Beer's law is shown to be applicable. The absorption peak is at 432 ± 2 mμ; extinction per milligram/liter is .0499 at this wave length, and the extinction ratio E_{420}/E_{450} mμ = 1.10 ± .005.

Application is made of these data to the determination of pure dye in commercial samples of the color. Typical results are given.

REPORT ON SUBSIDIARY DYES IN D&C COLORS
D&C RED Nos. 6 or 7

By L. Koch (H. Kohnstamm & Co., Inc., Brooklyn, N. Y.),
Associate Referee

Previous investigation of this subject by the Associate Referee,[1] and by collaborators,[2] has indicated the feasability of estimating the percentage of 4-toluene-azo-2-naphthol-3-carboxylic acid, in D&C Red No. 6, by titration with titanium trichloride. Continued work on this topic unearthed the fact that the method was not applicable to rosinated colors, because the extender masked the true end point.

A reduction type of assay was therefore considered, based on a modification of a procedure outlined in a previous paper.[3] Three certified specimens of rosinated D&C Red No. 7, with and without adulteration, were thereupon submitted to the following collaborators; K. C. Johnson, E. I. duPont de Nemours & Co., Wilmington, Del.; R. Pasternack, Chas. Pfizer & Co., New York, N. Y.; and A. T. Schramm, National Aniline Division, Allied Chemical & Dye Corp., Buffalo, N. Y. Their results are outlined in Table 1, and the Associate Referee wishes to express his indebtedness to them.

METHODS

REAGENTS

Stannous chloride soln.—100 grams of $SnCl_2$ per 100 ml of conc. HCl soln.

Sodium hydroxide soln.—50%.

Hydrochloric acid.—Approximately 0.3 *N*.

Potassium bromide-bromate soln.—0.05 *N* containing 1.3920 grams C.P. $KBrO_3$ and 10 grams of C.P. KBr per liter.

Sodium thiosulfate soln.—12.5 grams $Na_2S_2O_3 \cdot 5H_2O$ per liter. Standardize against $KBrO_3$–KBr soln as follows: Place 100 ml of water, 25 ml conc. HCl, and 100 grams of ice into an iodination flask. Add 20 ml of $KBrO_3$–KBr soln from a buret as rapidly as possible, and let stand in an ice bath for 10 min. Continue as directed under Procedure. Calculate the value of the $Na_2S_2O_3$ soln in the terms of the $KBrO_3$-KBr.

Starch indicator.—0.5% soln.

PROCEDURE

Weigh 5.0 g of sample into a beaker, and add 50 ml of glacial acetic acid, 25 ml of 6 *N* HCl, and 5 ml of the $SnCl_2$ soln. Cover the beaker with a watch-glass, and boil gently until the volume is ca 40 ml. Dil. the reaction mixture with 60 ml of methyl cellosolve, and boil again to half volume. Dil. the reduction product with 200 ml of water, cool and transfer to a 500 ml volumetric flask. Filter off a 200 ml aliquot, using Whatman #2, and place it in a 500 ml extraction funnel. Add 100 ml of water, and make the sample alkaline with ca 35 ml of 50% NaOH soln. Extract the liberated amine with two 100 ml portions of ether (if a non-separable emulsion forms

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[1] *This Journal*, 25, 948 (1942).
[2] *Ibid.*, 28, 763 (1945).
[3] Koch, *This Journal*, 29, 237 (1946).

add a few ml of methyl cellosolve to the supernatant ether layer and swirl gently). Wash the combined ether extracts with one 75 ml and three 40 ml portions of water.

Remove the amine from the ether with four 30 ml portions of ca 0.3 *N* HCl, and finally with 30 ml of water, transferring the washings to a 500 ml iodination flask, marked at the 100 ml level. Boil to expel dissolved ether, and conc. to 100 ml. Cool, and add 25 ml concd. HCl and ca 100 g of crushed ice. Add the $KBrO_3$-KBr soln from a buret, while agitating the flask, until the soln remains yellow at least a half-minute. Then add ca 5 ml more. Stopper the flask, and let it stand in an ice bath for 10 min. Add 2–3 g KI, and titrate while cold with the $Na_2S_2O_3$ soln, using the starch indicator internally only near the end point.

1 ml of 0.05 *N* $KBrO_3$ soln is equal to 4.11 mg of subsidiary dye—Na
1 ml of 0.05 *N* $KBrO_3$ soln is equal to 4.07 mg of subsidiary dye—Ca/2

TABLE 1.—*Collaborative results (per cent)*

ANALYST	SAMPLE 1		SAMPLE 2		SAMPLE 3	
A	0.18		0.95		3.48	
	0.17		0.90	0.93	3.47	
	0.18	0.18			3.18	
					3.23	
					3.40	3.35
B	0.02		0.67		3.02	
	0.02	0.02[1]	0.50	0.59	3.17	3.10
C		0.24		1.00		3.28
Ass. Referee		0.29		0.98		3.47
Calc.		0.24[2]		1.22[3]		4.01[3]

[1] Omitted in calculating average of results for Sample No. 1.
[2] Average subsidiary dye content of Sample No. 1.
[3] Average of results for Sample No. 1 plus the percentage adulteration.

COMMENTS BY COLLABORATORS

A. T. Schramm.—(1) Allow the first ether extraction to stand approximately two hours to facilitate separation.

(2) Add glass beads to the beaker before reduction, and during the evaporation of the ether to reduce the amount of "bumping."

DISCUSSION

The consistent low results obtained by the collaborators and the Associate Referee indicated that the reduction did not yield a quantitative recovery of p-toluidine. Experiments were therefore run to ascertain the percentage recovery of amine by treating 1 gram of pure subsidiary dye according to the proposed method. The results are shown in Table 2 in terms of subsidiary dye. Because it was also possible that large quantities of D&C Red No. 7 might alter the findings, 1 gram of subsidiary dye and

4 grams of certified color were also reduced and assayed for p-toluidine. The recoveries are outlined in Table 3.

TABLE 2.—*Results in terms of subsidiary dye*

SUBSIDIARY DYE		
TAKEN	FOUND	RECOVERY
mg	*mg*	*mg*
76.8	65.5	85.3
48.0	40.3	84.0
19.2	17.5	91.1

TABLE 3.—*Results assayed for p-toluidine*

SUBSIDIARY DYE		
TAKEN	FOUND	RECOVERY
mg	*mg**	*per cent*
76.8	67.0	87.2
48.0	40.6	84.6
19.2	17.5	91.1

* Minus the subsidiary dye content of the D&C Red No. 7.

Conclusions, based on these tentative experiments, indicate that the reduction conditions of the method do not give quantitative yields of the expected product. If these findings can be confirmed by additional work, a correction factor should be applied to the results obtained by this method, or a variation of the procedure should be made in order to obtain more quantitative recoveries of the amine.

It is therefore recommended* that the study of this topic should be continued.

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* For report of Subcommittee B and action by the Association, see *This Journal*, 31, 48 (1948).

WEDNESDAY—MORNING SESSION

REPORT ON FEEDING STUFFS

By L. S. **Walker** (Agricultural Experiment Station, Burlington, Vt.), *Referee*

RECOMMENDATIONS*

(1) It is recommended that further study be made on the following:

Mineral mixed feeds (calcium and iodine)
Lactose in mixed feeds
Fat in fish meal
Adulteration of condensed milk products
Crude fat or ether extract
Activity of yeast
Microscopic examination of feeds
Fluorine
Protein evaluation in fish and animal products
Hydrocyanic acid glucosides
Sampling and analysis of condensed buttermilk
Tankage (hide, hoof, horn, and hair content)

(2) It is recommended that the methods for calcium and phosphorus and the acetone method for fat in fish meal, as outlined by the Associate Referees, be made tentative and work continued.

(3) It is recommended that the editorial changes in the method for crude fiber, as recommended by the Associate Referee, be made, and study continued.

(4) It is recommended that work on the following be discontinued:

Fat in cooked animal feeds containing cereals
Crude protein (catalysis)

No report was given on mineral mixed feeds (calcium and iodine); or lactose in mixed feeds.

REPORT ON FAT IN FISH MEAL

By **Maurice E. Stansby** (Fish and Wildlife Service, Department of the Interior, Seattle, Washington), *Associate Referee*

Tests carried on in laboratories of the Fish and Wildlife Service during the past ten years have shown that when the fat content of fish meal is determined by conventional methods, that is, by extraction with ethyl ether, the apparent fat content drops during even short storage periods; while after six months to a year values obtained are as low as 30 per cent of the original. It has also been shown that when acetone is substituted for ethyl ether much higher recoveries of the initial fat are obtained, although a small drop in the apparent fat content does take place after extended storage periods. More recent studies have shown that certain mixed solvents give somewhat higher results than is obtained with acetone, but other difficulties in technique have thus far prevented the use of such mixed solvents on a routine basis. Inasmuch as such extremely low values are obtained when using conventional methods, it seemed advisable to test the acetone-extraction method on a collaborative basis even though it has not been perfected. Accordingly, during the past year such a collaborative analysis has been conducted.

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* For report of Subcommittee A and action of the Association, see *This Journal*, 31, 41 (1948).

Before starting the collaborative analysis it was necessary to standardize on a procedure. In preliminary experiments carried out during the past years it had been customary to first extract the meal with acetone then to evaporate off the acetone and redissolve the fat in ethyl ether. This solution was then evaporated and the ethyl ether-soluble material weighed as fat. This purification step was carried out in the past to be certain that the acetone was not dissolving extraneous material and that the final reported value was true fat. Extensive experience with this procedure, however, showed that the gross unpurified acetone extract of old meals was never higher than the purified ether extract of the same meal in the fresh condition nor was it higher than the initial fat content as determined by standard A.O.A.C. procedure. Thus, once having shown that the acetone extract contained no significant amount of extraneous materials it was felt that for a routine analysis it would be unnecessary to carry out the so-called "purification" step, and that by merely weighing the crude acetone extract a minimum value would be obtained which in no case would be higher than the true fat content of the meal. Accordingly, no purification with ethyl ether was included in the final procedure as adopted for the collaborative analysis.

Another decision which had to be made was whether a second step, namely, hydrolysis of the extracted meal with acid followed by a second extraction, should be included in the standardized method. Previous tests had shown that a considerable amount of additional fat could be extracted from meals in this way, especially meals which had been stored for a considerable length of time. Such a hydrolysis and extraction procedure on fresh meals usually yielded about 1 per cent additional fat by this method. For very old meals a considerably larger proportion of the meal was not extracted by the initial solvent but could be obtained by this hydrolysis procedure. Accordingly, in order to determine more nearly the true fat content in older meals it was decided to include the acid hydrolysis in the standard procedure. By so doing, the initial fat content of fresh meals is increased by about 1 per cent over values which would have been obtained had this process not been included.

The acetone procedure for fat in fish meal, exactly as sent to collaborative laboratories for this analysis is published in *This Journal*, **31**, 98 (1948).

FAT DETERMINATION—A.O.A.C. METHOD

Determine oil content by the official A.O.A.C. method for grain and stock feeds, as described on page 408 of the sixth edition of *Methods of Analysis*, section 27.24 and 27.25, first drying the meal as described under section 27.3. It should be noted that in drying to constant weight, ordinarily, a truly constant weight will never be attained with fish meal, because loss in weight due to evaporation of moisture, and gain of weight due to oxidation of fish oils (even in vacuum oven), both occur simultaneously. Drying is therefore discontinued after the sample ceases to lose weight.

Average results obtained in the collaborative analysis are shown in Table 1, while individual determinations in triplicate, as reported by the different laboratories, are given in Table 2. The meal used in this collaborative assay was an experimental pilchard meal prepared[1] in the pilot plant of the Seattle Fishery Technological Laboratory of the Fish and Wildlife Service, on September 25, 1946. The meal was stored in a paper bag at room temperature under conditions similar to those prevailing in the storage of commercial meals. A large sample was withdrawn during the latter part of April 1947, ground, and mixed thoroughly to insure homogeneity; and samples were placed in glass-stoppered paraffined

TABLE 1.—*Average values for fat in fish meal as reported by collaborative laboratories*

LABORATORY	ACETONE EXTRACTION METHOD			A.O.A.C. ETHYL ETHER METHOD
	INITIAL EXTRACT	ACID HYDROLYSIS EXTRACT	TOTAL EXTRACT	
	Per cent	*Per cent*	*Per cent*	*Per cent*
	Fat Content of Pilchard Meal Stored 7½ months			
A	11.15	2.57	13.72	5.25
B	13.32	2.57	15.67	4.92
C	11.9	3.7	15.6	5.8
D	11.0	2.1	13.1	4.57
E[1]	12.31	2.05	14.36	6.12
F	13.5	2.5	16.0	5.7
G	11.38	2.80	14.18	4.97
Average	12.08	2.58	14.66	5.33
	Initial fat content of freshly prepared meal			
	17.96	0.93	18.89	14.75[2]

[1] Soxhlet extraction equipment was used by Laboratory E.
[2] With hydrochloric acid digest extraction included, this value was 16.96 per cent.

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[1] The meal was prepared by a method somewhat similar to commercial wet process rendering but on a small scale. This involved cooking the pilchard with steam, pressing out oil in a hydraulic press, and drying the meal. The latter step differed from commercial practise in that a tunnel drier was employed using a blast of air at 150°F.

bottles and mailed to collaborative laboratories with instructions that all tests should be run during the ten-day period of May 10 to May 20. As shown in Table 1 the average fat content of the meal had fallen from the original 14.75 per cent in the freshly prepared meal (when determined by the ether extract method) to an apparent value of 14.66 per cent when determined by the acetone method, but to a value of 5.33 per cent when determined by the ether extract method (A.O.A.C.).

There was no outstanding difference in precision between the A.O.A.C. method and the acetone method either in regard to reproducibility between the triplicate samples run within the same laboratory or with respect to differences between individual laboratories. As a rule, individual laboratories were able to check themselves among the triplicate determinations better than they checked each other and this was true with both the acetone extraction method and the A.O.A.C. method. While absolute differences were greater in the case of the acetone extraction method, relative per cent differences were about the same. Values about three times as high were obtained for the acetone extraction fat determinations as for the A.O.A.C. method, and differences between replicate samples and individual laboratories were likewise about three times as high. Thus, the relative difference was of the same order of magnitude for the two different methods. On the whole, the precision was not nearly as good as could be desired, and future studies should be carried out to determine the cause for such lack of precision, and, if possible, to improve upon the procedure in order to obtain better agreement.

TABLE 2.—*Precision attained by individual laboratories on acetone and ethyl ether extraction of fish meal*

LABORATORY	FAT CONTENT OF MEAL			
	INITIAL EXTRACT	ACID HYDROLYSIS	TOTAL EXTRACT	A.O.A.C. ETHYL ETHER EXTRACT
Number	*Per cent*	*Per cent*	*Per cent*	*Per cent*
A	11.21; 11.27; 10.98	2.75; 2.32; 2.65	13.96; 13.59; 13.63	5.17; 5.27; 5.33
B	13.10; 13.18; 13.68	2.05; 2.77; 2.24	15.15; 15.95; 15.92	4.93; 4.90; 4.92
C	10.8; 12.0; 11.9	4.0; 3.5; 3.7	14.8; 15.5; 15.6	6.0; 5.7; 5.8
D	11.2; 10.6; 11.0	2.1; 2.1; 2.1	13.3; 12.7; 13.1	4.55; 4.70; 4.60
E	12.24; 12.17; 12.51	1.99; 2.08; 2.09	14.23; 14.25; 14.60	6.00; 6.20; 6.07
G	11.14; 11.48; 11.51	3.00; 2.72; 2.67	14.14; 14.20; 14.18	4.93; 4.98; 5.00

In spite of the fact that the acetone extraction method does not give ideal and complete extraction of fat from old meals and precision is not all that could be expected, the results by this method are so far superior to those obtained by ethyl ether extraction that it is felt the acetone method should be adopted tentatively, pending improvements which may require a considerable number of years to be attained. Accordingly, the following recommendations are made:

(1) It is recommended* that the acetone extraction procedure proposed be made tentative.

(2) That study be continued to improve the precision of the acetone extraction procedure.

(3) That studies be continued on the use of other solvents or mixtures of solvents which might eventually lead to a better procedure than is possible by the use of acetone.

LIST OF COLLABORATIVE LABORATORIES

Commonwealth of Virginia, Department of Agriculture and Immigration, Division of Chemistry, Richmond, Virginia

Maine Agricultural Experiment Station, Orono, Maine

State of California Department of Agriculture, Sacramento, California

State of Michigan Department of Agriculture, Lansing, Michigan

U. S. Department of the Interior, Fish and Wildlife Service, Fishery Technological Laboratory, Seattle, Washington

Wirthmore Research Laboratory, Malden, Massachusetts

No report was given on adulteration of condensed milk products.

REPORT ON FAT IN COOKED ANIMAL FEEDS CONTAINING CEREALS (ACID HYDROLYSIS)†

By STACY B. RANDLE (New Jersey Agricultural Experiment Station, New Brunswick, N. J.), *Associate Referee*

The Associate Referee has continued the investigation of the acid hydrolysis method for fat in cooked animal feeds containing cereals. Previously, it was shown (1, 2, 3) in individual and collaborative studies by the author that this method may give higher fat results than does direct ether extraction. Furthermore, it has been pointed out that extreme care must be exercised in order to obtain duplicable results by the acid hydrolysis method. Several other difficulties, such as emulsion formation, filtration of the sample, and separation of ether extract and hydrolysate, have been reported.

Schall and Thornton (4) have shown that not all the material extracted by this method is fat. The findings of the Associate Referee are in agreement with this report. It is apparent that the solvents used in the procedure may extract non-fatty materials. In view of the fact that this method is more tedious and requires greater skill of operation than the direct ether extraction method, your Associate Referee feels that the acid hydrolysis method would not receive widespread use, even tho the accuracy of the two methods were the same.

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* For report of Subcommittee A and action of the Association, see *This Journal*, 31, 41 (1948).

† Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, Department of Chemistry.

It is the opinion of the Associate Referee that the present acid hydrolysis method is not so reliable as the direct ether extraction method for the determination of fat in dog feeds. There are more difficulties encountered in the acid hydrolysis procedure. It is admittedly true that both methods determine ether extract and not true fat. It is recommended,† therefore, that the acid hydrolysis method be not adopted for the determination of fat in cooked animal feeds containing cereals and that further investigation of the method for this purpose be discontinued.

REFERENCES

(1) *This Journal*, **25**, 864, 1942.
(2) *Ibid.*, **26**, 340, 1943.
(3) *Ibid.*, **28**, 768, 1945.
(4) *Ibid.*, **26**, 404, 1943.

No report was made on crude fat or ether extract, or on activity of yeast.

REPORT ON MICROSCOPIC EXAMINATION OF FEEDS

By H. J. WITTEVEEN (Department of Agriculture, Dairy and Food, St. Paul, Minn.), *Associate Referee*

The last report on the microscopic examination of feeds to this Association was made by Mr. A. W. Creswell of the Ohio Department of Agriculture in 1944. Based on a survey made about that time, microscopic examination of feeds was started in several States in 1907. This work continued to expand so that by 1942, 24 States and the Dominion of Canada employed full or part time microscopists. Included in the survey was an outline of a procedure, together with the necessary equipment.

Early this year this study was resumed by sending letters to twenty-four feed control officials and chemists associated with feed manufacturing concerns requesting that an outline of their procedure be submitted, stating the preliminary steps and describing the characteristic appearance of each ingredient identified. Sixteen replies were received, five of which included procedures. In addition to the five who submitted procedures two stated that microscopic work was being done; procedures were not submitted but interesting and helpful comments were made.

The request for the description of the characteristic appearance of each ingredient identified resulted in a variety of comments. The majority stated that studying reference samples and acquiring experience was the most satisfactory way of learning how to identify ingredients. Simplicity in procedure was expressed as an important factor particularly where many samples are examined and a limited amount of time can be devoted to each sample. Others submitted procedures and outlines describing the characteristic appearance of the various feed ingredients.

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† For report of Subcommittee A and action of the Association, see *This Journal*, **31**, 41 (1948).

Of interest in the study of this problem is the work of the Check Sample Committee of the Association of the American Feed Control Officials. During the early part of the year a sample of feed was prepared containing ten ingredients common to most feeds, nine of which were included in a list of fifteen comparable to the ingredients listed on a tag or label. Portions of the sample were sent to forty-five collaborators, thirty-one of whom were control chemists, the other being chemists associated with feed manufacturing concerns. Each collaborator was supplied with a copy of the following procedure and a description of the characteristic appearance of each of the fifteen ingredients listed as stated in "Microscopical Examination of Feeds and Feedingstuffs" by B. H. Silberberg (1918), revised by Geo. L. Keenan (1940).

PROCEDURE FOR IDENTIFICATION OF FEED INGREDIENTS

Prepared by the A.A.F.C.O. Collaborative Check Sample Committee

As a preliminary to the actual identification of the numerous ingredients contained in present day mixed feeds, each analyst should provide himself with the proper equipment for the work, including a microscope, hand lenses, slides, cover glasses, needles, sieves, scalpels, solutions, and authentic type samples of the various ingredients commonly contained in mixed feeds. The following references, and others not mentioned, are also of great importance:

The Microscopy of Vegetable Foods, by A. L. Winton.
The Structure and Composition of Foods, by A. L. and K. B. Winton.
Elementary Chemical Microscopy, by Chamot.
Massachusetts Agricultural Experiment Bulletin 141.
Special Bulletin 120 Agricultural Experiment Station, East Lansing, Mich.
Bulletin 246 Vermont Agricultural Experiment Station, Burlington, Vermont.

The next step is for the analyst to thoroughly familiarize himself with the appearance of the various feed ingredients without magnification and with magnification of such degree as he feels will enable him to recognize numerous ingredients both singly and mixed with other ingredients. For practice, mixtures of various ingredients should be made up by the analyst and identification studied. Labels showing manufacturers' statements of ingredients contained are of assistance to the analyst in providing leads of what to look for. In cases where an ingredient is claimed and not possible of identification, a minor amount (one or two per cent) of the missing ingredient might be added from an authentic source, to determine if such small amounts can be identified in the mixture under examination.

The following is a modification of the preliminary procedures used in the Maryland and Minnesota Feed Control laboratories on mixed feeds (especially those containing added mineral constituents).

Sift a portion (ca 10 g) of the unground mixed feed on a 20-mesh sieve. The material remaining on the sieve is placed on a white paper or glass and examined under a strong light, with a hand lens or microscopically, or both. The portion which passes thru the sieve may be used to verify the first findings by examining under the microscope, using chloral hydrate soln (45 g chloral hydrate, 25 ml hydrochloric acid (1–8), with 10 ml of glycerine) as a clearing agent.

A second portion of 10 g of the unground feed is mixed with 50 ml of chloroform in a 100 ml beaker. After stirring with a spoon allow to settle briefly and skim off the floating portions containing most of the feed. Pour off the excess chloroform. After drying on a low-heat hot plate to evaporate the chloroform, examine the settlings which contain the mineral ingredients in fairly pure condition. Individual chemical tests should be made for chlorides, carbonates, sulphates, iron, copper, iodine, phosphates, and grit, as specified in A.A.F.C.O. Official Publication; the A.O.A.C. *Methods of Analysis* (6th Ed.); and other standard references.

These collaborators were asked to identify the ingredients listed and any other ingredients they might find.

Results Obtained in the Microscopic Examination of Prepared Feed

INGREDIENTS DECLARED	31 CONTROL CHEMISTS		14 INDUSTRIAL CHEMISTS	
	FOUND BY—	PER CENT	FOUND BY—	PER CENT
Ground Corn	31	100.0	14	100.0
Ground Oats	24	77.5	14	100.0
Corn Gluten Feed	23	74.2	13	92.9
Alfalfa Meal	23	74.2	10	71.5
Cottonseed Meal	25	80.6	10	71.5
Soybean Oil Meal	22	71.0	10	71.5
Linseed Meal	26	83.8	10	71.5
Wheat Bran	31	100.0	14	100.0
Iodized Salt	31	100.0	14	100.0
Defluorinated Phosphate	18	58.1	9	64.2
INGREDIENTS NOT DECLARED				
Calcium Carbonate	19	61.3	13	92.9
Brewers Grains	6	19.4	5	35.7
Weed and Grass Seeds	6	19.4	3	21.4
Ground Barley	12	38.7	7	50.0
Meat Scraps	10	32.2	5	35.7
Coconut Meal	5	16.1	4	28.6
Peanut Meal	6	19.4	3	21.4

The data in the above table indicate that five control chemists and one industrial chemist made perfect analyses. A high percentage of perfect analyses would be unlikely inasmuch as feeds are impure products, small amounts of ingredients being present as impurities or as generally occurring with a product of similar nature, such as oats and barley, bran and middlings, etc.

In this connection familiarity with macroscopic characteristics might be helpful in avoiding identification of ingredients present as impurities or accompanying ingredients of a similar nature such as those just mentioned. To identify ingredients from their macroscopic appearance would also reduce the amount of time required for each sample.

Thus far little consideration has been given to the identification of mineral ingredients and whole weed seeds in feeding stuffs. Further study including these phases of the work would be desirable.

It is recommended* therefore that the study of microscopic examination of feeds be continued.

No report was given on fluorine in feeding stuffs.

MINERAL CONSTITUENTS OF MIXED FEEDS

By J. L. St. John (*Associate Referee*) and Edith Eng Huey
(Division of Chemistry, Agricultural Experiment Stations,
and State Chemist's Laboratory, Pullman, Washington)

Following last year's report (*This Journal*, **30**, 606 (1947)), additional samples of the identical feed samples previously utilized were sent to a second group of collaborators. The methods used are described in the previous report.

Since only summarized averages were reported last year, the results obtained by individual collaborators for both 1946 and 1947 are included in Table 1.

The results of the two years' work are so nearly the same that the conclusions of last year are not changed. The results obtained by the nitric-perchloric acid method of sample preparation are essentially the same as those given by the A.O.A.C. method **12.5**. It thus appears that these two methods of sample preparation for calcium and phosphorus in mixed feeds may be used interchangeably. The nitric-perchloric acid method of sample preparation is therefore recommended† as tentative. The details of the procedure are published in *This Journal*, **31**, 98 (1948).

Precautions regarding the use of perchloric acid to guard against the danger of an explosion, which has been feared by analysts, are described in the previous paper, (*This Journal, loc. cit.*) and in *Ind. Eng. Chem. Anal. Ed.*, **13**, 48; **14**, 301; **16**, 630. It is emphasized that all of the easily oxidizable organic matter should be oxidized with nitric acid before the perchloric acid is added.

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* For report of Subcommittee A and action of the Association, see *This Journal*, **31**, 41 (1948).

† For report of Subcommittee A and action of the Association, see *This Journal*, **31**, 41 (1948).

TABLE 1.—*Comparison of results by two methods*

COLLABORATORS	CALCIUM IN FEEDS						PHOSPHORUS IN FEEDS					
	SAMPLE 1		SAMPLE 2		SAMPLE 3		SAMPLE		SAMPLE 2		SAMPLE 3	
	A.*	B.	A.	B.	A.	B.	A.	B.	A.	B.	A.	B.
	Per Cent		*Per Cent*		*Per Cent*		*Per Cent*		*Per Cent*		*Per Cent*	
Bartlett, L. E.	1.96	2.01	2.25	2.22	2.06	2.11	0.924	0.956	1.076	1.096	1.124	1.120
Beaver, B. F.	1.97	1.97	2.22	2.17	2.04	2.01	0.777	0.670	0.945	0.914	0.975	0.970
Despaul, J. E.	1.91	1.98	2.09	2.22	1.99	2.01	0.795	0.805	0.935	0.915	0.965	0.943
Fuqua, V. L.	1.75	1.66	1.94	1.94	1.84	1.75	0.76	0.57	0.91	0.75	0.95	0.76
Haskins, A. L.	1.96	2.05	2.20	2.20	2.06	2.08	0.76	0.79	0.91	0.89	0.95	0.93
Hayes, C. M.	2.01	2.02	2.21	2.26	2.11	2.08	0.911	0.862	1.018	1.058	1.054	1.112
Jarvis, N. D.	2.32	1.90	2.49	2.15	2.22	1.97	0.97	0.83	0.93	0.92	1.16	0.94
Kinney, C. N.			1.99	2.18	1.99	2.11			0.95	0.79	1.02	0.83
Koskoski, F. J.	2.01	2.03	2.11	2.19	2.05	2.07	0.81	0.78	0.94	0.95	0.99	0.96
Struve, O. I.	1.98	1.95	2.18	2.16	2.02	2.06	0.79	0.79	0.97	0.95	0.97	0.97
Huey, E. E.	2.02	1.98	2.21	2.06	2.05	1.97	0.82	0.83	0.80	0.94	0.83	1.00
Randall, F. E.	2.05	1.91	2.23	2.10	2.11	1.97	.81	.82	.93	.94	1.00	1.00
Midkiff, V. C.	2.00	2.00	2.17	2.17	2.06	2.10	.79	.78	.93	.93	.97	.97
Hunter, W. L.	1.99	1.98	2.19	2.17	2.05	2.04	.803	.796	.950	.940	.988	.969
Geagley, W. C.	1.96	1.96	2.17	2.19	2.05	2.05	.78	.79	.91	.92	.94	.95
Fritz, J. C.	2.00	2.00	2.28	2.27	2.14	2.13	0.87	.88	1.02	1.02	1.06	1.07
Overholser, J. S.	1.96	1.96	2.20	2.15	2.03	2.05	.81	.81	.94	.94	.97	1.01
Fudge, J. F.	2.28	2.82	2.47	2.93	2.32	2.75	1.34	.89	1.57	1.19	1.65	1.17
Elmslie, W. P.	2.12	2.01	2.27	2.22	2.08	2.09	.78	.79	.95	.94	.98	.98
Merrill, E. C.	2.22	2.41	2.49	2.60	2.19	2.39	0.71	0.76	0.83	0.90	0.86	0.99
Ingram, W. J.	2.00	2.02	2.17	2.16	2.08	2.03	0.84		0.97		1.01	
Average	2.02	2.03	2.22	2.22	2.07	2.09	0.84	0.80	0.96	0.95	1.02	0.98

* A—A.O.A.C. Methods of Analysis (6th Ed., p. 415 (27.47), p. 127 (12 37 (b).
B =Nitric-perchloric Method (*Ind. Eng. Chem. Anal. Ed.*, 7, p 167 (1935).

REPORT ON CRUDE FIBER

By WILLIAM L. HUNTER (State of California, Department of Agriculture, Sacramento 14, Calif.), *Associate Referee*

The 1946 report on the determination of crude fiber contained a preliminary discussion of a survey of the method as applied by the several participants in the collaborative sample of the Association of American Feed Control Officials. It was observed that the present procedure was but rarely followed in all details.

The diversity of techniques reported is extremely disconcerting in the instance of a method which is as openly arbitrary and empirical as that for crude fiber. This is especially true in some cases where the variations depart from the official method almost completely. It seems necessary to warn all laboratories interested in crude fiber that departure from the official method in any respect places them in a vulnerable position in an action based on crude fiber analyses. It may be argued that the results obtained by a variation of the method do not represent crude fiber content in a legalistic sense and are, therefore, immaterial and irrelevant. This possibility is not too far-fetched since crude fiber is not a specific chemical substance, but rather the result of an arbitrary physico-chemical test. It is the residue, the insoluble material remaining after a series of carefully prescribed operations. Liberties taken with the procedure may alter the results and defeat agreement between laboratories. Strict adherence to the established method cannot be too strongly emphasized. It should be followed in all respects.

While insisting upon adherence to the official method, it appears that some revisions of this procedure are in order for the purposes of clarity, preciseness, and emphasis of detail. The use of Erlenmeyer flasks, which are now prescribed for the digestion, has given way to the use of beakers almost unanimously.

The present allowance for final filtration through either alundum crucibles or filter cloth is in an opposite category. Study of results of laboratories using these has indicated that they may be involved in unsatisfactory results. The alundum crucibles are difficult to wash free of the alkaline extract, and the use of cloth involves excessive handling. Revisions are recommended which may be regarded as editorial.[1]

It is recommended*—

(a) That study of the crude fiber determination be continued to allow for investigation of possible revisions as well as those necessary in the case of materials which are high in mineral.

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[1] The changes suggested have been published (*This Journal*, 31, 99 (1948)).

* For report of Subcommittee A and action of the Association, see *This Journal*, 31, 41 (1948).

REPORT ON CRUDE PROTEIN IN FEEDING STUFFS (CATALYSTS)

By RODNEY C. BERRY (State Div. of Chemistry, Department of Agriculture, Richmond, Va.), *Associate Referee*

According to the recommendation of the Association at the 1946 meeting and under the approval of the General Referee on Feeding Stuffs, a collaborative study on Crude Protein in high concentrate feeding stuffs, with special reference to catalysts, was inaugurated during the year.

Samples of cottonseed meal, fish meal, and meat scrap were submitted to 15 collaborators (13 of whom completed the work, with one too late to tabulate) who were instructed to make analyses in triplicate according to four methods, *viz:* (1) *Methods of Analysis*, sec. **2.24** using both cupric sulfate and mercuric oxide (2) sec. **2.25** cupric sulfate only (3) sec. **2.26,** using mercuric acid only, and (4) using selenized granules along with approximately 0.7 grams of mercuric oxide, 30 ml concentrated sulfuric acid and 10 grams of potassium sulfate. Selenized granules were furnished by the Referee.

Criticisms of the methods were requested and the collaborators were very generous with their cooperation; some of them also submitted results based on their own methods or other variations in procedure.

COLLABORATORS

(A) Walker, L. S., Chemist-in-charge, State Agricultural College, Burlington, Vermont.

(B) Randle, Stacy B., New Jersey Agri. Experiment Sta., New Brunswick, N. J.

(C) Smith, John B., R. I. State College, Kingston, R. I.

(D) Marshall, Chas., Plant Production Div., Dept. of Agri., Ottawa, Canada.

(E) Hunter, Wm. L., State Dept. of Agri., Sacramento 14, California.

(F) Geagley, W. C., Michigan Dept. of Agriculture, Lansing, Michigan.

(G) Randall, Fred, Cooperative G. L. F. Mills, Inc., Buffalo, New York.

(H) Etheredge, M. P., State College, Miss.

(I) Bides, P. R., Auburn, Alabama, 515 Dexter Avenue.

(J) Koskoski, Frank J., N. Y. State Agri. Exp. Sta., Geneva, New York.

(K) Burns, Loren V., M. F. A. Milling Co., Springfield, Missouri.

(L) Berry, Rodney C., 1123 State Office Building, Richmond, Virginia. Shuey, Phillip McG., c/o Shuey & Co., 115 E. Bay St., Savannah, Georgia.

RESULTS AND DISCUSSION

A complete tabulation of results is submitted in the tables along with criticisms of the methods.

The average clearing time for all four methods on all samples was shorter when selenium was used, varying from 12 to 50 minutes. The use of mercury alone (method 3) was preferred by the majority of collaborators. Some analysts prefer selenium-oxychloride alone and selenium and copper sulphate for their routine analyses.

TABLE 1.—*Collaborative results on crude protein in feeding stuffs*

COLLABORATOR	SAMPLE 1. C. S. MEAL				SAMPLE 2. MEAT SCRAP				SAMPLE 3. FISH MEAL			
	METHOD				METHOD				METHOD			
	Cu-Hg	Cu	Hg	Se-Hg	Cu-Hg	Cu	Hg	Se-Hg	Cu-Hg	Cu	Hg	Se-Hg
	1	2	3	4	1	2	3	4	1	2	3	4
A	35.86	36.01	36.60	36.07	46.92	47.38	47.90	47.95	58.84	58.56	60.19	60.59
B	36.69	36.94	37.38	37.06	48.31	47.46	49.13	49.09	60.46	60.52	61.29	61.42
C	37.31	36.64	37.54	36.84	48.58	48.66	49.56	49.11	60.76	61.88	61.85	61.30
D	36.10	35.50	36.50	36.30	47.90	46.40	47.70	47.80	59.80	58.10	59.60	59.70
E	36.6	37.4	37.6	37.5	48.4	48.8	48.8	48.6	60.6	61.0	61.4	61.4
F	35.9	36.48	36.96	35.79	47.52	47.34	48.48	47.54	59.38	60.34	60.67	59.75
G	36.85	36.95	36.92	37.15	48.05	48.25	48.92	48.82	60.48	60.58	61.15	61.32
H	36.73	37.21	37.23	37.11	48.48	48.67	48.81	48.67	60.56	61.40	61.29	61.25
I	36.66	37.19	37.19	37.19	48.54	48.54	48.75	48.85	60.21	60.62	61.66	61.04
J	36.50	37.50	37.30	36.90	48.60	49.00	48.60	48.30	61.20	59.30	61.20	61.40
K	35.74	36.56	36.53	36.58	47.17	48.37	48.24	48.33	59.51	60.69	60.59	60.60
L	37.88	38.25	38.38	38.13	49.13	49.06	49.50	49.38	62.25	62.44	61.75	61.56
Av.	36.24	36.88	37.17	36.88	48.13	48.16	48.70	48.53	60.33	60.45	61.05	60.94
Av. Total		36.88				48.38				60.70		
Dev. from Av.	−.64	+.005	+.297	+.005	−.25	−.22	+.32	+.156	−.363	−.15	+.35	+.244
Diff. High & Low	1.09	2.75	1.88	2.34	2.21	2.66	1.86	2.84	3.41	4.34	2.25	1.86
#3 over #1			+.937				+.57				+.713	
No. High	none	1	9	2	1	3	7	2	none	4	3	5
No. Low	9	2	none	1	7	4	none	1	9	3	none	none

The percentage recovery of protein as compared with the average of all four methods seems to be in the order: mercury, selenium and mercury, copper, copper and mercury, with the greatest difference on the sample of cottonseed meal. There was only a slight difference in recovery between mercury alone and mercury and selenium, when applied to meat scrap and fish meal.

TABLE 2.—*Collaborative comments on crude protein in feeding stuffs*

	#1—C.S. MEAL METHOD				#2—MEAT SCRAP METHOD				#3—FISH MEAL METHOD				
	1	2	3	4	1	2	3	4	1	2	3	4	ANALYST
Clearing time in Minutes	100	80	60	45	45	50	35	30	50	60	40	30	(A) E. F. Boyce
	75	80	60	50	75	80	85	50	85	90	70	50	(B) Ralph L. Willis
	No significant difference. Prefers Method #3												(C) R. W. Gilbert
	24 Choice #3 Also run by Se-Oxychloride—				Choice #1				Choice #1 preferred				(D) Dr R. Payfer
	No comments												(E) V. E Entwistle C. A. Luman
	25	24	17	14	Clearing effect due to the ratio of acid and K_2SO_4								(F) Percy O'Meara
	25	30	18	12	Slight preference for Selenium.								(G) F. E. Randall
	Prefers #3—Mercury												(H) A. G McKee
	Method #4 is faster.												(I) P. R. Bides
	19	35	25	13	17	19	22	13	15	21	17	13	(J) F. J Kokoski
	Digests 3.5 hours Also ran 3 additional methods Prefers $CuSO_4$—Se.												
	20	30	15	15	#1—is violent on addition of water. #3—tends to crystallise.								(K) R. L. Matthews
	20	20	20	20	Same comments as above.								(L) W. J. Franklin

The digestion time varied from one hour after clearing to three hours. There was considerable difference in results among collaborators on each method, with a high low ratio of 4.34% in method (2) on fish meal.

RECOMMENDATIONS*

In view of the results obtained by this study and also according to results obtained in previous work on nitrogen in soils, plant material, and fertilizers (see references), it is recommended that A.O.A.C. Method **2.26** for nitrogen be made preferential when applied to protein materials in feeding stuffs containing 30% or more, and that further work on catalysts in protein determination be discontinued.

REFERENCES

Vickery, *This Journal*, **29**, 358 (1946).

Lauro, M. F., *Indus. Eng. Chem., Anal. Ed.*, **3**, 401–402 (1931).

Osborn, R. A., and Krasnitz, A., *This Journal*, **16**, 110 (1933).

Prince, A. L., *Soil Science*, **59**, 1 (1945).

———, *This Journal*, **24**, 264–268 (1941).

———, *Ibid.*, **26**, 54 (1943).

———, *Ibid.*, **17**, 246 (1934).

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* For report of Subcommittee A and action of the Association, see *This Journal*, **31**, 41 (1948). Details of official changes are given on page 99 (*loc. cit.*).

No reports were given for protein evaluation in fish and animal products on hydrocyanic acid glucosides, or on sampling and analysis of condensed buttermilk.

APPOINTMENTS

K. G. Clarke, Division of Fertilizer and Agricultural Lime, Department of Agriculture, Beltsville, Md., has been appointed as Associate Referee on Inert Materials (Fertilizers).

CONTRIBUTED PAPERS

THE PRESENT STATUS OF THE ANTIMONY TRICHLORIDE (BLUE-COLOR) PROCEDURE FOR THE ESTIMATION OF VITAMIN A IN MARGARINE[1]

By E. E. RICE,* E. PRIMM,† A. I. COOMBES‡

When the program of enrichment of margarine with vitamin A was initiated several years ago, the search for a rapid, precise, and accurate method of analysis for this vitamin was intensified. From the practical viewpoint of control laboratories, an acceptable method must be capable of yielding accurate and reproducible results in the hands of skilled technicians. It should be adaptable to different types of margarines without extensive modifications, yet simple enough to permit workers in different laboratories to obtain concordant values.

In general, there are three types of methods which can be used for the determination of vitamin A: biological, spectrophotometric, and colorimetric.

1. *Biological assays for vitamin A*, while fundamentally essential to prove the validity of any chemical or physical method, are obviously unsuited for the routine testing of large numbers of samples, being slow, expensive, and lacking in precision.

2. *Spectrophotometric methods*, under certain conditions, can be used to advantage. In general, there are three approaches, each of which has certain limitations.

(a) The vitamin A content may be determined from the difference in optical density at 328 mμ between the unfortified margarine oil and oils separated from the melted, fortified margarine. This method is restricted to cases where the unfortified oils can be obtained, since oil mixtures differ sufficiently to necessitate a blank for each run. However, when the unfortified oils are available, the method is rapid and precise.

(b) The sample may be saponified and the vitamin A content determined from the difference between the optical densities of the unsaponifiable fraction before and after irradiation with ultraviolet light to destroy the vitamin (1). This method is much more complicated than (a), requiring careful control of analytical conditions and the use of especially purified solvents. Furthermore, the unsaponifiable materials other than vitamin A must be stable to ultraviolet irradiation.

(c) The sample may be saponified and purified by chromatography before the determination of the optical density at several wave lengths in the 300–350 mμ region. There are several such methods, each of which requires rather complicated manipulations. For example, that of Wilkie and DeWitt (2) involves calculations based on the measurement of vitamin A at an optical density other than that of the maximum. On a theoretical basis, this latter point is sufficient to cause most analysts to pause before undertaking the method. In practice, the scheme has proved successful in the hands of some analysts, but has failed completely for others. A relatively new method has been advocated by Awapara, Mattson, Mehl, and Deuel (3). With this technique, the optical density of a benzene solution of the unsaponifiable fraction of a margarine is measured before and after specific adsorption of vitamin A on Floridin, and the vitamin A in the sample is calculated from the difference between the observed optical densities.

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[1] This paper is a report of the work of the Subcommittee on Vitamin A, National Association of Margarine Manufacturers.

* Swift & Company, Chicago, Ill.

† John F. Jelke Company.

‡ Wilson & Co., Inc.

3. *Chemical methods* are generally based upon the development and measurement of the blue color formed by treatment of vitamin A with antimony trichloride. Most of these methods are complicated by the need for preparing an extract of the unsaponifiable material in margarine. While vitamin A is ordinarily the only substance occurring in margarine which yields the blue color with antimony trichloride, other substances sometimes affect the rate of development and the intensity of the color, thus requiring the use of correction factors. With care, the method yields reproducible values which check biological assays within the limits of error of the latter method.

In the hope that a unified program might bring about more rapid advances than individual efforts, a number of investigators interested in vitamin A methods met in 1944 and thoroughly discussed the problems involved and the best plan for solving them. This meeting initiated a series of collaborative studies of the various methods and modifications.

Initially the blue-color method, essentially as applied by Dann and Evelyn (4), was most commonly used and seemed most promising for development. However, there was no agreement regarding the exact conditions necessary for best results, and little data regarding the precision and accuracy of the method when applied to margarine. Accordingly, replicate samples of several margarines, and of the oils and vitamin concentrates going into them, were distributed for analysis by methods in use by the various laboratories. These investigations were directed toward determinations of the conditions necessary for most efficient and effective application of the various operations involved in blue-color methods. After consideration of the data obtained in two preliminary collaborative studies, a blue-color procedure was developed embodying the combined experience of the group. In a third and fourth collaboration, this method has been compared with two modifications of the spectrophotometric technique and its application to a variety of margarines has been studied. The following report presents the data obtained in these studies and some of the viewpoints of the collaborators.

METHODS

(1) *Distribution of Samples.*[1]—Samples of the oils to be used in the manufacture of margarine were removed from the mixing vats prior to addition of vitamin A concentrates. The manufacturing processes, including enrichment with known quantities of vitamin A, were then com-

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[1] By John F. Jelke Company, Armour and Company, Durkee Famous Foods, Kraft Foods Company, Swift and Company, and Mrs. Tucker's Foods, Inc.

pleted and fifty one-pound prints of each margarine were taken directly from the packaging lines. Two prints, 100 ml of the mixed unfortified oils, and several capsules of a high-potency vitamin A ester concentrate[2] for use as a standard were shipped to:

Sadie Brenner, QM Food & Container Institute for the Armed Forces
Francis W. Chornock and Eldon E. Rice, Swift & Company
A. Irving Coombes, Wilson & Co., Inc.
H. J. Deuel, Jr., J. W. Mehl, and J. Awapara, University of Southern California
Stephen Fein, Monica Cunningham, and Delores Gehrs, Kraft Foods Company
Carl S. Ferguson, Massachusetts Dept. of Public Health
C. Gilmore, Durkee Famous Foods
J. A. Mathews, Distillation Products, Inc.
Kenneth Morgareidge, Nopco Chemical Company, Inc.
Ralph H. Neal, The Best Foods, Inc.
Bernard Oser, Food Research Laboratories, Inc.
Elizabeth Primm, John F. Jelke Company
John F. Roland, The Armour Laboratories
Lawrence Rosner and Gerda L. Siegel, Laboratory of Vitamin Technology
Arthur H. Seibert, Shilstone Testing Laboratory
Harold K. Steele, The Fleischmann Laboratories
Fred W. Wharton, Mrs. Tucker's Foods, Inc.

(The above alphabetical arrangement does not correspond with the numerical order in the tables; these numbers have been assigned in the order in which the reports were received in the third collaboration).

(2) *Analytical Procedures.*—Each collaborator was requested to assay the margarine in duplicate by one or more of the following methods.

A. Blue Color Method[3]

(1) Quarter a print, mix thoroly, and weigh a 10.0-g portion into a 250–350 ml flask.

(2) Add 50 ml of alcoholic alkali (10 g of KOH per 100 ml of soln; specially denatured No. 30 alcohol is satisfactory) and reflux for 15 mins.

(3) Cool, add 100–150 ml of water, and extract successively with 125-, 100-, and 75-ml portions of peroxide-free diethyl ether.

(4) Pool the extracts and wash once with 100 ml of water, once with 50 ml of 0.5 *N* KOH or NaOH, and then with successive 75- to 100-ml portions of water until free from alkali (wash soln colorless to phenolphthalein).

(5) Allow to stand 15 min., drain out the last droplets of water, and shake the ether extract with 3–5 g anhydrous sodium sulfate. Filter and wash the extraction vessel and sodium sulfate twice with 25-ml portions of ether.

(6) Remove the ether by evaporation *in vacuo* or in an atmosphere of nitrogen.

(7) *Immediately,* dissolve the unsaponifiable material in chloroform and dilute to a volume such that the expected vitamin concentration is in the proper range for the instrument to be used in measuring the intensity of the blue color. This concentration is usually 5–15 units per ml.

(8) Place 1 ml of this solution in a cuvette, add 1 ml of chloroform, then add rapidly 10 ml of antimony trichloride reagent (25 g reagent grade $SbCl_3$ dissolved in 100 ml $CHCl_3$) and *immediately* determine the per cent transmission at 620 mμ.

Determine M, the U.S.P. units of vitamin A represented by this transmission reading, from a reference curve correlating per cent transmission with various known quantities of the vitamin prepared by direct dilution with chloroform of a weighed quantity of U.S.P. Vitamin A Reference Standard.[4] M, multiplied by the dilution factor (total volume of chloroform solution of margarine unsaponifiables divided by the weight of margarine), gives the potency of the margarine in terms of units per gram.

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[2] Obtained from Distillation Products, Inc.

[3] This method is essentially that published by the Association of Vitamin Chemists, Inc. (5), which includes more detail regarding preparation of reagents and suggestions for avoiding or correcting difficulties.

As a check upon the accuracy of this value it is desirable to make an additional reading and an increment calculation.

(9) To another 1-ml portion of the soln of unsaponifiables add 1 ml of a chloroform soln containing S, a known number of units of vitamin A. (Prepared by dilution of the U.S.P. Reference Standard.) For greatest precision, S should be approximately equal to M. Add rapidly 10 ml of reagent and *immediately* determine the per cent transmission at 620 mμ. Determine I, the units of vitamin A represented by this transmission reading, from the reference curve.

It is now possible to use an increment technique for determining the vitamin A potency of the margarine by relating M and I to the known value of S. This relation may be stated:

$$\text{Units per ml of dilution} = \text{M} \div \frac{(\text{I}-\text{M})}{\text{S}}$$

This equation introduces [(I−M)/S] as a correction factor to compensate for any inhibition of blue color formation by impurities in the sample under test. In effect, [(I−M)/S] is the ratio between the recovery of a known quantity of vitamin A in the presence of possible interferences and the known value, S.

In place of M and I, their corresponding per cents transmission may be converted to optical densities (optical density = 2 − log per cent transmission) and substituted in the above equation. S, in this case, is still expressed in units of vitamin A per ml.

Such calculations do not take into account the color of the test solution or of colors formed from non-vitamin A materials upon treatment with the reagent. Ordinarily, corrections for these extraneous colors are unnecessary. However, in order to make certain that such colors would not affect precision in the third collaboration, each collaborator was requested to prepare a blank by saponifying 8.1 g[5] of the unfortified oil and to determine the blank B. (B is again expressed as units of vitamin A per ml from the standard curve, or as optical density calculated from the observed per cent transmission.) Determination of the concentration of vitamin A in the solution of the unsaponifiable fraction of the margarine was than made from the formula:

$$\text{Units per ml of dilution} = (\text{M}-\text{B}) \div \frac{(\text{I}-\text{M})}{\text{S}}$$

B. Blue Color Method on Whole Margarine Oils (no saponification).

Melt slowly a portion of the margarine and allow the oil to separate. Filter through rapid paper. Using this clear oil and the untreated unfortified oil (or $CHCl_3$ dilutions of them), determine values corresponding to M, B, S, and I, and calculate the potency as indicated in A.

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[4] This reference curve should be checked at frequent intervals to minimize variations due to changes in reagents and manipulation. At the time of the collaboration this standard was not available and oil of high potency, supplied by Distillation Products, Inc., was used as a reference.

[5] Margarines contain approximately 81 per cent of oil mixtures.

C. Spectrophotometric Method on Whole Margarine Oils.

Prepare oil from the margarine as in Section B. Dilute with cyclohexane as required and determine the optical density at 325–8 mμ, using a similar concentration of the unfortified oil as a blank. Calculate potency from the formula (O.D./C × 2000 × 454 × 0.81 = units per pound of margarine, where O.D. = observed optical density, C = concentration of oil in grams per 100 ml of soln, and 2000 is the factor for converting O.D./C into units of vitamin A. (Using the new U.S.P. Vitamin A Reference Standard, the conversion factor is 1894).

D. The Method of Wilkie and DeWitt (2, 8).

E. The Method of Awapara, Mattson, Mehl, and Deuel (3).

In the third collaborative study of vitamin A methods, each member of the committee was supplied with two pounds of product from a single manufacturer, and with a sample of the unfortified oils from which the margarine had been made. Each analyst was requested to assay the samples by as many of the methods as possible. The values obtained are summarized in Table 1.

TABLE 1.—*Vitamin A analyses reported for a margarine sample—third collaboration*

(Thousands of units per pound)

LAB. NO.	DATE OF ASSAY 1946	SPECTROPHOTOMETRIC TECHNIQUE*	BLUE COLOR TECHNIQUES*†			
		UNFORTIFIED OIL vs. OIL SEPARATED FROM MARGARINE	VALUES OBTAINED DIRECTLY FROM CALIBRATION CURVE	VALUES OBTAINED BY INCREMENT TECHNIQUE	VALUES FOR UNSAPONIFIED OIL, BY INCREMENT TECHNIQUE	BLANK VALUES, FROM CALIBRATION CURVE
1	2–5		14.3 E	16.1		
	2.7		17.2 E	19.7		
	Avg.	17.8 B	15.7	17.9		0.5
2	2–6		17.3 E	18.1		
	2.6		16.5 E	17.5		
	Avg.	17.4 B	16.9	17.7	18.6	.5
3	2–6		15.6 E	15.3	Impossible (values?)	.0
4	2–8		14.5 E	15.4		
	3–27		15.5 E	16.5		
	Avg.		15.0	15.9		.5

TABLE 1.—(*continued*)

LAB. NO.	DATE OF ASSAY 1946	SPECTROPHOTOMETRIC TECHNIQUE*	BLUE COLOR TECHNIQUES*†			
		UNFORTIFIED OIL *vs.* OIL SEPARATED FROM MARGARINE	VALUES OBTAINED DIRECTLY FROM CALIBRATION CURVE	VALUES OBTAINED BY INCREMENT TECHNIQUE	VALUES FOR UNSAPONIFIED OIL, BY INCREMENT TECHNIQUE	BLANK VALUES, FROM CALIBRATION CURVE
5	2–8		16.4 C	16.1		
	2–8		17.4 C	15.9		
	Avg.	17.4 B	16.9	16.0	16.2	.6
6	2–16	16.3 G	16.3 E	17.4	17.8	.3
7	2–26		15.1 E	16.6		
	2–26		15.0 E	16.2		
	Avg.		15.0	16.4	—	.8
8	2–27		16.7 E	16.4	—	.3
9	3–4	15.5 C	19.0 C	18.2	—	1.6
10	2–20		16.6 E	17.8		
	3–4		14.8 E	15.4		
	3–6		15.4 E	15.7		
	Avg.	16.8 B	15.6	16.3	15.4	—
11	3–8		17.5 C			
	3–8		17.6 C			
	Avg.		17.5			
12	3–10	20.3 B	17.7 E	18.1		.5
13	2–18	16.4 B				
14	2–13		15.4 E	15.0		
	Avg.	17.2 (16.8)	16.4	16.7	17.0	.5
Standard Deviation		1.4 .78	1.2	1.1		
Coefficient of Variation		8% 5%	7%	7%		

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* The types of instruments used in determining the vitamin content of the various solutions are indicated by the letters B, C, E, and G, these referring to the Beckman spectrophotometer, the Coleman spectrophotometer, the Evelyn photoelectric colorimeter, and the Gaertner spectrograph, respectively.

† Each value recorded in the table is the average of duplicate analyses. In no case did these duplicates differ by more than 6% of their mean, and in most cases the differences were less than 4%.

After meeting and discussing these data, the Committee arranged for a fourth collaboration. Six different manufacturers sent samples to the committee members, who were asked to assay each sample by the blue color method and to report values representing direct readings from a standard curve, and also values representing the calculation M÷(I−M)/S. The Floridin technique (3) and modification of the glycerol dichlorhydrin method (6) were also to be used where possible. The manufacturers were requested to supply products with a vitamin A content either slightly higher or slightly lower than their normal production to provide a range of expected potencies. Several types of oils and vitamin concentrates were used in preparing the margarines, the combinations being:

Manufacturer I, soybean oil plus vitamin A esters

Manufacturer II, mixed vegetable oils, plus vitamin A esters

Manufacturer III, mixed vegetable oils plus vitamin A alcohol

Manufacturer IV, 75 per cent oleo oil and 25 per cent vegetable oils plus vitamin A esters

Manufacturer V, mixed vegetable oils, rich in cottonseed oil, plus vitamin A esters

Manufacturer VI, mixed vegetable oils, plus vitamin A esters.

Data obtained in this fourth collaboration appear in Tables 2 and 3.

DISCUSSION

The transient nature of the blue color and the unequal rates of fading of the color in standard and test solutions, coupled with differences in techniques for making the galvanometer readings, preclude expectation of perfect checks between laboratories or even within a single laboratory. In view of these difficulties, the variations in values reported by blue-color methods (Tables 1 and 2) are not surprising, even though they do leave much to be desired insofar as precision is concerned.

Because the analytical manipulations are few and simple, greater precision might be expected when values are obtained by the spectrophotometric technique in which oil from fortified margarine is compared with the unfortified oil blend from which the margarine was made. Actually, the values found by this technique were disappointing, their range being even greater than that for the blue-color assays (column 3, Table 1). It is of interest, however, that the low value obtained by this method (15.5 by Laboratory 9) was obtained with a Coleman spectrophotometer with readings from a standard curve; and the high value (20.3 by Laboratory 12) was obtained with a Beckman spectrophotometer by separate readings of E1%/1 cm for blank oil and fortified oil. In the other laboratories, where Beckman or Gaertner instruments were used and in which method C was followed, the agreement was much better, the values ranging from 16.3 to 17.8.

Blue-color values found by interpolating galvanometer readings upon recently checked calibration curves usually agreed reasonably well with

TABLE 2.—*Blue color analyses reported for various margarines—fourth collaboration*
(Thousands of units per pound)

LAB. NO.	SOURCE OF SAMPLE											
	MANUFACTURER I		MANUFACTURER II		MANUFACTURER III		MANUFACTURER IV		MANUFACTURER V		MANUFACTURER VI	
	A^1	B^2	A^1	B^2	A^1	B^2	A^1	B^2	A^1	B^2	A^1	B^2
(Fortification Level)	21.1	21.1	17.0	17.0	18.8	18.8	19.2	19.2	18.0	18.0	16.5	16.5
1	17.9	20.7*	13.8	15.6	16.4	18.0	16.2	17.7	15.9	17.2	14.7	16.0
2	19.6	22.7	15.8	17.8	18.4	21.3	17.1	20.4	17.4	20.1	16.2	18.4
3	17.6	20.1*	13.6	15.3*	16.0*	17.6*	16.0*	17.9*	15.7	17.0*	14.4	16.5*
5	18.9	18.1	15.5	15.7	17.6*	18.0	18.0*	17.9	17.0	18.1	16.1	16.4
6	19.3	19.9	15.1	15.6	17.4	17.8	17.6	18.0	16.8	17.1	15.6	16.0
7	18.3	19.8	14.3	15.4	16.5	18.2	16.4	17.9	16.4	17.7	14.7	15.8
10	18.7	21.2	13.8	15.5	16.2	17.9	16.2	17.0	14.6	15.2	13.9	15.0
11	18.7	17.9*	15.1	15.1	17.6	18.2*	17.6	16.7	17.1*	17.4*	15.9	16.3*
13	17.7	18.1*	14.1	15.3	15.6	16.7	17.0*	17.1*	15.6	15.9*	14.1	14.1
14	18.7	20.5	14.7	15.3	17.2	17.9	17.6	18.5	16.9	18.4	15.7	16.3*
15	21.7	22.5	16.9	16.7	19.6	20.1*	19.5*	20.0*	18.7*	19.9*	14.7	16.6
16†	(17.8)	(17.3)	(13.4)	(13.1)	(14.3)	(13.1)	(15.5)	(17.8*)	(15.9)	(17.1*)	(13.6)	(14.1)
17	18.2		12.6		15.8		13.8		14.5		14.5	
Avg.	18.7	20.1	14.6	15.8	17.0	18.3	16.9	18.1	16.4	17.6	15.0	16.1
Standard dev.	1.2	1.2	1.2	0.6	1.1	1.4	1.4	1.1	1.1	1.4	1.1	1.1
Coef. of var.	6.3	7.9	8.1	7.0	6.6	7.4	8.5	5.6	5.9	7.6	6.9	6.8

[1] Values obtained by interpolation of values upon a previously prepared reference curve.
[2] Values obtained by an increment calculation.
* Poor checks: spread of 1000 units per pound or more.
† Not included in averages because subsequent data show instruments used to be in need of adjustment.

those found by an increment technique (Table 1, columns 4 and 5; and Table 2 columns A and B). However, in general, the increment calculations indicate a vitamin A content 4 to 8 per cent greater than do calculations based on readings from a calibration curve; this despite the fact that the former entails a correction for the blank while the latter does not. Occasional assays have been reported in which the increment technique results in values as much as 20 per cent greater than those read from reference curves. This trend has been observed previously for a number of types of margarine, and brings into question the use of a blank value in calculations.

TABLE 3.—*Per cent recovery of vitamin A added to unsaponifiable material of margarines as determined by blue color analyses**

LAB. NO.	FOURTH COLLABORATION						LABORATORY MEAN	STANDARD DEVIATION
	MANUFACTURER NO.—							
	1	2	3	4	5	6		
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		
1	88	88	90	90	92	90	89.7	1.5
3	88	89	91	89	92	87	89.3	1.9
4	98	92	94	99	98	100	96.8	3.1
5	104	99	98	100	94	100	99.2	3.3
6	97	97	97	98	98	97	97.3	.2
7	92	93	91	92	92	93	92.2	.2
10	88	89	91	95	97	93	93.2	3.5
11	105	99	97	105	99	98	100.5	3.6
14	94	93	90	94	91	94	92.7	1.7
15	96	101	98	98	94	89	96.0	4.2
16	102	102	110	87	93	97	98.5	8.0
Mean	95.6	94.7	95.1	95.1	94.6	94.4		
Standard Deviation	6.3	5.0	5.9	5.3	2.9	4.3		

* These figures represent the per cent recovery of a known amount of vitamin A added to $CHCl_3$ solutions of the unsaponifiable of the various margarines. Each figure is the average of two values

The blanks used in obtaining the data shown in Table 1 were measured by assaying the unfortified oil blend from which the margarine was made. Theoretically, the use of such a blank is the most valid procedure. Practically, however, the unfortified oils are seldom available and blanks of this nature cannot be obtained. When the unfortified oils are available, calculations should be made by an increment technique to compensate for inhibitions of color development by materials present in the unsaponifiable fraction.

Another type of blank has been reported (7), this being a color-blank which is estimated by diluting the test solution with chloroform instead of with reagent, and reading the per cent light transmission the same as for the sample. In either case the readings for the blank are small, and relatively small instrument errors cause large errors in the blank. Also the color-blank does not compensate for decreases or increases in color which may occur when reagent is used, but which do not occur in chloroform. The increment values shown in Table 1 were calculated from the formula $(M-B)\div[(I-M)/S]$=units per ml of dilution; those in Table 2 were calculated from the formula $M\div[(I-M)/S]$ units per ml of dilution.

The existence of an inhibitory effect is amply demonstrated by the data in Table 3, which show recoveries averaging 94 to 96 per cent of vitamin A added to the unsaponifiable materials of the fortified margarines of both collaborations. In many cases the decrease in vitamin A potency caused by this inhibition is almost the same as the value for the unfortified oils; thus, direct readings from a calibration curve check closely with calculations by the more complicated increment technique. This observation has led many analysts to question the validity of a blank correction. In the case of the sample used in collaboration No. 3 it is rather apparent that subtraction of blanks from the values reported by calculations from a calibration curve (Table 1, Column 4) would result in values in poorer agreement with those obtained by the several other methods. This has been observed in previous collaborations and in many observations reported in committee meetings. In fact, it has been so general as to lead to the tacit assumption by some analysts that the blank compensates for inhibition of color formation, and that values may be calculated directly from a calibration curve without consideration of the blank. For routine work with margarine or oils of known composition, this technique reduces manipulative error and greatly simplifies calculations. With unknown materials the assays should always be checked by recovery experiments, and increment calculations must be made if the recoveries are lower than 95 per cent. It should be realized that the use of readings from a curve without the use of a proper blank is not a scientifically sound method. since it involves compensating errors.

While the increment technique is theoretically the most sound, it exaggerates deviations in blue-color readings, since an error made in M also results in an erroneous value for $I-M$. Thus, if the value for M is 1 per cent low, $I-M$ will be about 1 per cent high; and since M is divided by $I-M$, the answer will be approximately 2 per cent low instead of the 1 per cent of the initial value. Likewise, each increment calculation involves two values, M and I, and hence doubles the chance for error. Nevertheless the presence of inhibitory substances in many margarine extracts is sufficient to warrant the use of increment calculations. It should be noted that "increment results" in Table 1 are rather uniformly higher than those read from a curve. Random error would of course produce an equal number of high and low values.

The data presented in Table 2 give an erroneous impression of the precision of the methods so far as individual laboratories are concerned. By careful inspection of the data it may be seen that certain laboratories reported values which are consistently higher (or lower) than the mean. Thus, the values of Laboratories 2 and 15 are 5 to 15 per cent higher than the means for the various samples, while those for Laboratories 16 and 17 are low by similar amounts. Other laboratories (notably Nos. 6, 7, and 14) report values consistently near the means. This indicates good precision within the individual laboratories, but failure of all laboratories to obtain the same level of values. The experiences of several laboratories indicated the quality of the antimony trichloride used in preparing the reagent to be a factor influencing these interlaboratory variations.

The extension of blue-color assays by increment technique to include unsaponified oils (Method B) seems an interesting possibility. In the third collaboration, four laboratories were able to get reasonable values (Column 6, Table 1). Only one reported failure of this method. In the fourth collaboration, five laboratories reported values by this technique in agreement with the regular blue-color assays.

Considerable difficulty was reported in conducting assays according to the procedure of Wilkie and DeWitt. In the third collaboration, eight laboratories attempted the procedure. Three failed completely and the other five reported erratic values. Data for four (Nos. 1, 2, 10, 13) have been reported in detail by Wilkie (8) as Nos. 2, 7, 19, and 4, respectively. Laboratory 5, in a number of attempts, obtained but one value of 18,000 units per pound. Even those laboratories reporting values have had difficulties in obtaining consistent adsorption on the chromatographic columns as discussed by Wilkie (8). In general, the opinion of many analysts working with this latter method has been that it is too long and the technique is too involved for satisfactory analyses. Two types of correction factors are involved: (1) the use of a factor to correct for incomplete recoveries, and (2) the use of optical densities determined at 340 mμ rather than at 325–30 mμ. Failure of the method when optical densities are measured at the point of maximum absorption for vitamin A constitutes a serious fundamental drawback. This becomes of special significance when attempts to recover vitamin A by recognized procedures fail. Three laboratories conducting analyses by the chromatographic adsorption technique also conducted blue-color assays of solutions which had been chromatographed. The blue-color values were only 10,000–12,000 units per pound (60–75 per cent of the amounts found by the regular blue-color procedure). In fact, in repeated attempts, one laboratory was unable to get blue-color assays of more than 10,000–12,000 units per pound when solutions of unsaponifiables were prepared according to the saponification and extraction technique recommended for the chromatographic procedure. Despite this, following adsorption and elution, values of 15,000–17,000 units were indicated by measurements at 340 mμ and calculations according to directions for the chromatographic method. This seems to indicate measurement of a modified form of vitamin A by the latter technique, and raises the question as to whether the method might measure this form of the vitamin if it occurred naturally. More data of this type are needed before the merits of chromatography can be evaluated fully.

In the fourth collaboration several laboratories attempted to apply the method of Awapara, Mattson, Mehl, and Deuel (3). This method recommends saponification of the sample, extraction of the vitamin A and part of the other unsaponifiables with Skellysolve A, washing of the extract with water, evaporation of the solvent followed by solution of the residue in benzene, and determination of the vitamin content from the optical density of the benzene solution before and after treatment with specially-prepared Floridin. The Floridin quantitatively adsorbs vitamin A and the difference in the optical density at 328 mμ between untreated and Floridin-treated extracts should be a measure of the vitamin content of the solution. Success of the method depends upon adjustment of extraction and adsorption techniques so that all of the vitamin is extracted from the saponification mixture and so that vitamin A is the only material absorbing light at 328 mμ which is retained by the Floridin column. In general, those laboratories using this technique have obtained high values for vitamin A, presumably because of the removal of materials other than vitamin A from the benzene extract by the Floridin. Refinements of the technique which are being developed by its proponents may eliminate this fault.

Modifications of the technique of Sobel and Werbin (6) have also failed for the several laboratories attempting to devise techniques for the adaptation of glycerol dichlorohydrin to margarine assays. The most common criticisms have been that the rate of color formation is different in margarine extracts than in standard solutions, and that the rate of fading is much more rapid for extracts than for standards.

SUMMARY AND CONCLUSIONS

Results have been presented of two collaborative studies in which replicate samples of margarine have been assayed by seventeen laboratories. In general, there has been better agreement among the values reported by blue-color analyses than among those obtained by the spectrophotometric methods now available. Direct calculation of the potency of the sample from values found by interpolation of the observed transmission upon a calibration curve agreed well with those obtained by a calculation involving a correction for observed recoveries of vitamin A, *i.e.*, by an increment type calculation. The coefficient of variation for blue-color values reported from thirteen laboratories is 7 per cent, with mean values of 16,425 units per pound (readings from a calibration curve) and 16,724 units per pound (increment calculations).

At present, most of the analysts who participated in this collaborative work believe the blue-color technique as outlined for increment calculation to be the most reliable general method available for margarine, but that, in most cases, readings may be made directly from a calibration curve.

BIBLIOGRAPHY

(1) NEAL, R. H., and LUCKMAN, F. H., "Determination of Vitamin A Content of Margarine." *Ind. Eng. Chem., Anal. Ed.*, **16**, 358 (1944).
(2) WILKIE, J. B., and DE WITT, J. B., "Spectrophotometric Procedure for the Estimation of Vitamin A in Oleomargarine." *This Journal*, **28**, 174 (1945).
(3) AWAPARA, J., MATTSON, F. H., MEHL, J. W., and DEUEL, H. J., JR., "A New Spectrophotometric Method for the Determination of Vitamin A." *Science*, **104**, 602 (1947).
(4) DANN, W. J., and EVELYN, K. A., "The Determination of Vitamin A with the Photoelectric Colorimeter." *Biochem. J.*, **32**, 1008 (1938).
(5) The Association of Vitamin Chemists, Inc., "Methods of Vitamin Assay." Interscience Publishers, Inc., N. Y. (1947).
(6) SOBEL, A. E., and WERBIN, H., "Determination of Vitamin A in Fish-Liver Oils with Activated Glycerol Dichlorohydrin—Comparison with Spectophotometric and Antimony Trichloride Methods." *Anal. Chem.*, **19**, 107 (1947).
(7) OSER, B. L., MELNICK, D., and PADER, M., "Estimation of Vitamin A in Food Products." *Ind. Eng. Chem., Anal. Ed.*, **15**, 724 (1943).
(8) WILKIE, J. B., "The Present Status of the Chromatographic Spectrophotometric Procedure for the Estimation of Vitamin A in Margarine." *This Journal*, **30**, 382 (1947).

ISOLATION AND SPECTROPHOTOMETRIC CHARACTERIZATION OF FOUR CAROTENE ISOMERS

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The presence of neo-beta-carotene U and neo-beta-carotene B has been demonstrated in "pure" carotene extracts from plant sources (1, 10). Since these isomeric pigments have reduced nutritional value (3, 4), it is evident that physiological activity as determined by beta-carotene analysis may be in considerable error if the relative amounts of the stereoisomers present are not taken into account. Quantitative spectral data for these isomers are meager. The scarcity of such information on neo-beta-carotenes U and B made it desirable to prepare these materials in pure crystalline form for use as standards in the determination of the individual stereoisomers after separating them from one another by the flowing chromatographic technique (2).

In a recent A.O.A.C. report on carotene (11), the Associate Referee recommended that the spectrophotometric method for carotene be tentatively discontinued and that further work be done on the absorption

TABLE 1.—*Specific absorption coefficients at highest maximum for all-trans-alpha- and all-trans-beta-carotene (taken from literature)*

SOLVENT	ALL-TRANS-ALPHA CAROTENE		ALL-TRANS-BETA CAROTENE		REF.
	WAVE LENGTH mμ	SPECIFIC ABSORPTION COEFFICIENT	WAVE LENGTH mμ	SPECIFIC ABSORPTION COEFFICIENT	
Petroleum ether, b.p. 63–70°C (Skelly Oil Co.)	—	—	450	238	(15)
Heptane	448	—	455	240	(18)
Petroleum ether (40–60°C.)	—	—	450	243	(15)
Petroleum ether (40–55°C.)	—	—	447	245	(17)
Iso-octane	444	252	450	245	(14)
Petroleum ether	445	255	450	249	(13)
Petroleum ether (60–80°C.)	—	—	452	250	(8)
Hexane	—	—	453	253	(5)
Petroleum ether, 63–70°C (Skelly Oil Co.)	—	—	450	253	(19)
Petroleum ether (55–65°C.)	445	279	450	254	(21)
Hexane (65–67°C.)	446	272	450	258	(24)
Hexane	—	—	450	259	(19)
Petroleum ether (62–65°C.)	445–6	272	452	259	(23)
Hexane	—	—	453	261	(5)
Hexane	—	270	450	276	(20)
Petroleum benzine	—	—	450	280	(7)
Hexane	—	273	—	302	(12)

coefficients, since carotene content of plant extracts did not agree at the three wave lengths prescribed by the method. A study of the literature revealed a wide variation in the specific absorption coefficients for all-trans-alpha and all-trans-beta-carotene (Table 1). In the present investigation these isomers were prepared in as pure a state as possible. The methods of isolation and purification and the specific absorption coefficients of these four carotene isomers are given. The effects of several hydrocarbon solvents upon specific absorption coefficients have been evaluated.

EXPERIMENTAL

Materials:

Adsorbent.—The adsorbent used to separate alpha- and beta-carotene from one another was a 1-to-1 mixture of Micron brand* magnesia (Westvaco Chlorine Products Co. No. 2642) and Dicalite,* (Dicalite Co. No. 4200): a diatomaceous earth (22). This mixed adsorbent was also employed for concentrating dilute solutions in preference to evaporation procedures.

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* The mention of these products does not imply that they are endorsed or recommended by the Department of Agriculture over others of a similar nature not mentioned.

The adsorbent used to separate the beta-carotene stereoisomers was Shell brand* lime, chemical hydrate, 325 mesh, as recommended by Polgar and Zechmeister (16) and used by us in previous work (2).

Carotene.—A commercial crystalline carotene (90% beta, 10% alpha) was employed as the starting material for the preparation of purified isomers. Isomerized carotene solutions were prepared by adding iodine (equivalent to 2% of the weight of the dissolved carotene) to the solution of purified beta-carotene in hexane about one hour before the solution was added to the chromatographic column.

Drying Agent.—Anhydrous crystalline sodium sulfate was used in all cases to dry the petroleum ether solutions prior to chromatographic adsorption.

Solvents.—For the development of the chromatograms on magnesia-Dicalite columns, 2% by volume of reagent-grade acetone in commercial hexane (Phillips Petroleum Co.)* was used.

For the development of the isomerized beta-carotene chromatogram on hydrated lime columns, either a 2% solution (by volume) of acetone in hexane or a 1½% solution (by volume) of para-cresyl methyl ether in hexane was used (2).

Other solvents used in this work were:

Methyl and ethyl alcohols, refluxed over magnesium and redistilled prior to use.

Chloroform, shaken with concentrated sulfuric acid, washed, dried, and redistilled.

Benzene, dried over sodium and redistilled.

Reagent-grade acetone, used without redistillation.

A low-boiling fraction of petroleum ether (37° to 43°C.), obtained by fractionation of a low-boiling commercial solvent.

The following materials, without any pretreatment, were used as solvents for carotene in the determination of absorption coefficients: isooctane (Rohm and Haas),* commercial hexane (Phillips Petroleum Co.), and three petroleum ethers having boiling point ranges as indicated in Table 3 (Skelly Oil Co.).*

For comparison with Zscheile's work, a special hexane fraction was prepared as described in his report (24) by treating the commercial hexane exhaustively with alkaline permanganate (about 3 weeks) and redistilling. Only the portion which distilled between 65° and 67°C. was used.

Chromatographic Apparatus:

The chromatographic tubes used were similar to those described by Zechmeister (22). No. VI tubes were used for the separation of isomers, No. II tubes for the concentration of solutions on magnesia-Dicalite, and No. I tubes for the analytical determination of the purity of the various preparations.

Spectrophotometric Measurements:

A Beckman quartz spectrophotometer, Model DU,* was used for absorption spectra measurements. The band width over the region 400 mμ–500 mμ was substantially constant at 0.5 mμ. In the region 320 mμ–400 mμ, the band width was less than 1.0 mμ, being about 0.6 mμ at 340 mμ. The absorption coefficients obtained with narrower spectral bands (0.3 mμ) in the region 400 mμ–500 mμ were identical with those presented. The wave length scale, as calibrated against the mercury arc spectrum, is within 0.2 mμ in the region covered in this study. There were no observable changes of the specific absorption coefficients for the carotene solutions during the interval required for obtaining the spectral values.

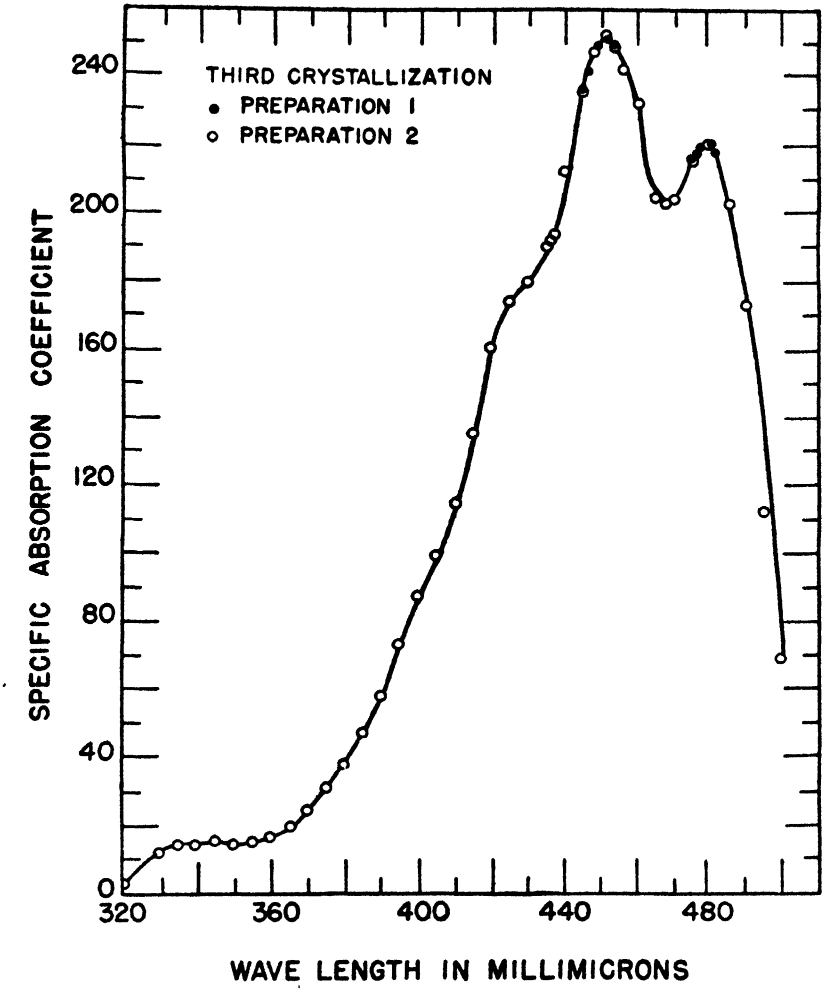

FIG. 1.—Absorption spectrum of all-trans-beta-carotene in iso-octane.

Purification of Pigments:

All-trans-beta-carotene.—900 ml of a hexane solution containing 0.5 g of commercial carotene (90% beta, 10% alpha) was added to a No. VI chromatographic column packed to a height of 28 cm with the magnesia-Dicalite mixture. The column was developed with a 2% solution of acetone in hexane. After the alpha- and beta-carotene bands had become sufficiently separated, the column was extruded, the alpha- and beta-carotene bands were individually carved out, and the pigments eluted with a 10% solution of alcohol in hexane. The solution was filtered, the alcohol removed by a continuous washing device (6), and the solution dried with anhydrous sodium sulfate. The beta-carotene solution was subjected to two additional chromatographic separations on magnesia-Dicalite to remove the last traces of alpha-carotene. After elution of beta-carotene from the last column, alcohol equivalent to 5% by volume was added to the hexane solution of carotene (150 mg carotene in 300 ml) and the solution stored under nitrogen at −30°F. After several hours, the crystals that had settled were filtered off, washed with methyl alcohol, and dried under vacuum at room temperature for 2 hours. Chromatographic adsorption analysis of a solution of these crystals on hydrated lime

TABLE 2.—*Wave length positions of maximum and minimum absorption, and corresponding specific absorption coefficient,*[1] *of carotene isomers in iso-octane*

	ISOMER			
	ALL-TRANS-ALPHA-CAROTENE	ALL-TRANS-BETA-CAROTENE	NEO-BETA-CAROTENE U	NEO-BETA-CAROTENE B
		Maximum		
Wave length, mμ	446	451	447	443
α-Preparation 1[1]	267–267	252–251	239–238	192–194
α-Preparation 2	267–269	252–251	237–237	187–189
Average	267	251	238	191
		Minimum		
Wave length, mμ	462	468	462	462
α-Preparation 1	184–184	203–203	184–185	149–151
α-Preparation 2	184–185	204–202	184–183	147–149
Average	184	203	184	149
		Maximum		
Wave length, mμ	474	479	474	470
α-Preparation 1	244–243	220–221	207–206	154–156
α-Preparation 2	245–245	220–221	205–206	150–150
Average	244	221	206	153

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[1] Specific absorption coefficient: optical density referred to solution depth of 1 cm and concentration of 1 g/liter.

showed only one homogeneous zone of the all-trans-beta form with no evidence of the presence of alpha-carotene, oxidation products, or cis isomers of the beta-carotene. A portion of this material was used immediately for the determination of specific absorption coefficients, and the remainder was dissolved in benzene and recrystallized with methyl alcohol. Specific absorption coefficients were again obtained after removal of the crystals by centrifugation, washing with methyl alcohol, and drying

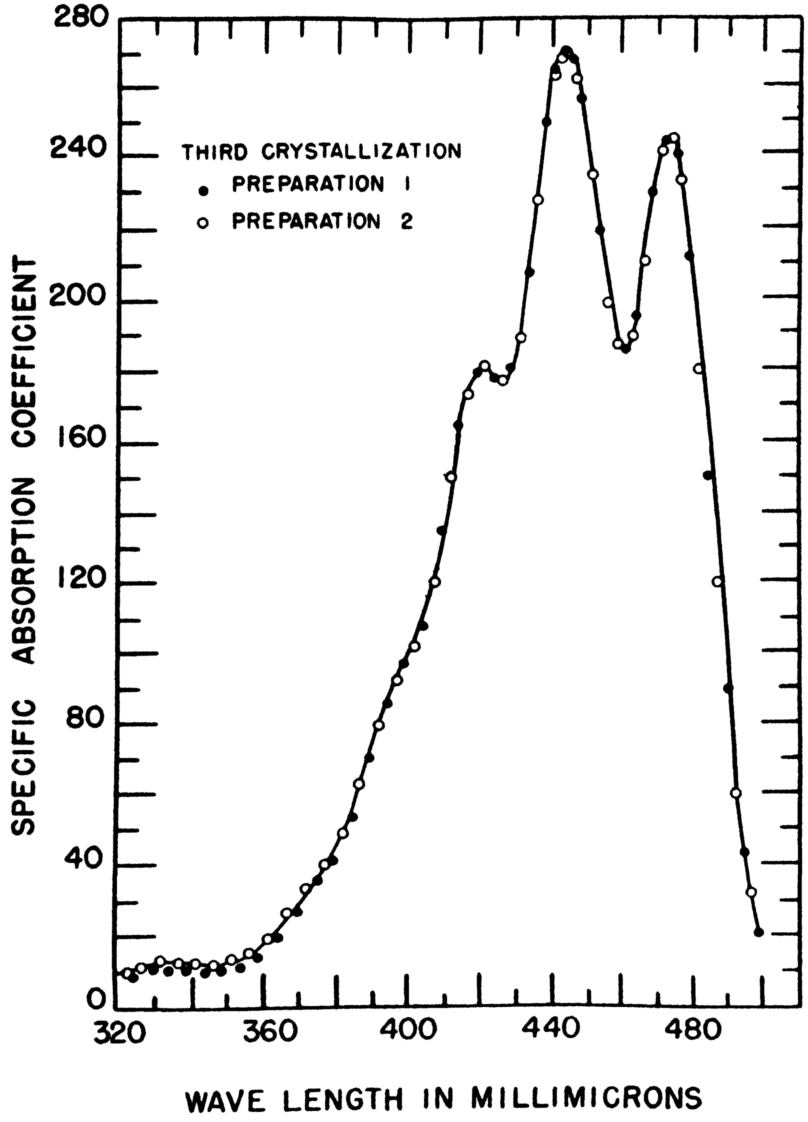

FIG. 2.—Absorption spectrum of all-trans-alpha-carotene in iso-octane.

as before. The material was subjected to two additional recrystallizations in the same manner, and specific absorption coefficients were obtained for each preparation. No increase in specific absorption coefficient was obtained after the first recrystallization (Figure 1 and Table 2).

All-trans-alpha-carotene.—The alpha-carotene was purified from the same source and with the same technique as described above for beta carotene (Figure 2, Table 2).

Neo-beta-carotene U.—Polgar and Zechmeister have described the preparation of this stereoisomer (16). An iodine-isomerized beta-carotene solution is chromatographed on a hydrated lime column, and after development with 2% acetone in petroleum ether, the homogeneous neo-beta-carotene U zone appears above and separated from the all-trans zone. It is carved out, eluted with ether, and the ether solution (which now amounts to several hundred ml) is evaporated to dryness. The pigment is then taken up in a minimum of benzene and the isomer is precipitated out upon addition of alcohol. When this procedure was followed in our laboratory, it was found that evaporation of the ether solution resulted in reisomerization of a portion of the neo-beta-carotene U isomer to the all-trans form even when precautions were taken to keep the solution cold and under an atmosphere of nitrogen at all times.

The procedure was therefore modified so that only one or two ml of solvent was evaporated. The purified isomer was eluted from the hydrated lime column with 20% alcohol in hexane instead of with ether. After the alcohol was removed by the continuous washing technique and dried, the pigment was readsorbed on a No. II column packed with the magnesia-Dicalite mixture. The minimum amount of adsorbent (1 g per 2.5 mg carotene) was used to retain all the carotene. After all of the pigment had been absorbed, 50 ml of the low-boiling fraction of petroleum ether (37°–43°C.) was drawn through the column. The pigment was then rapidly eluted with a solution containing equal volumes of acetone and ethyl alcohol. The eluate was washed free of acetone and alcohol and about 2 ml of the low-boiling petroleum ether (37°–43° C) remained, in which the carotene was now concentrated. This solution was filtered through an asbestos pad on a micro filter funnel into a 12-ml conical-type centrifuge tube and the solution was rapidly evaporated to dryness by bubbling a stream of nitrogen through it for 1 to 2 minutes. The pigment was taken up in a minimum of benzene and 3 to 4 volumes of methyl alcohol were added. When the solution was cooled in an ice-water bath, small needle-like crystals of neo-beta-carotene U precipitated out. The crystals were removed by centrifugation, washed several times with a minimum amount of methyl alcohol, and dried under vacuum at room temperature for one hour. A portion of this preparation was taken for absorption spectra determinations, and the remainder was recrystallized twice more. The absorption coefficients obtained from the three crystalline prepara-

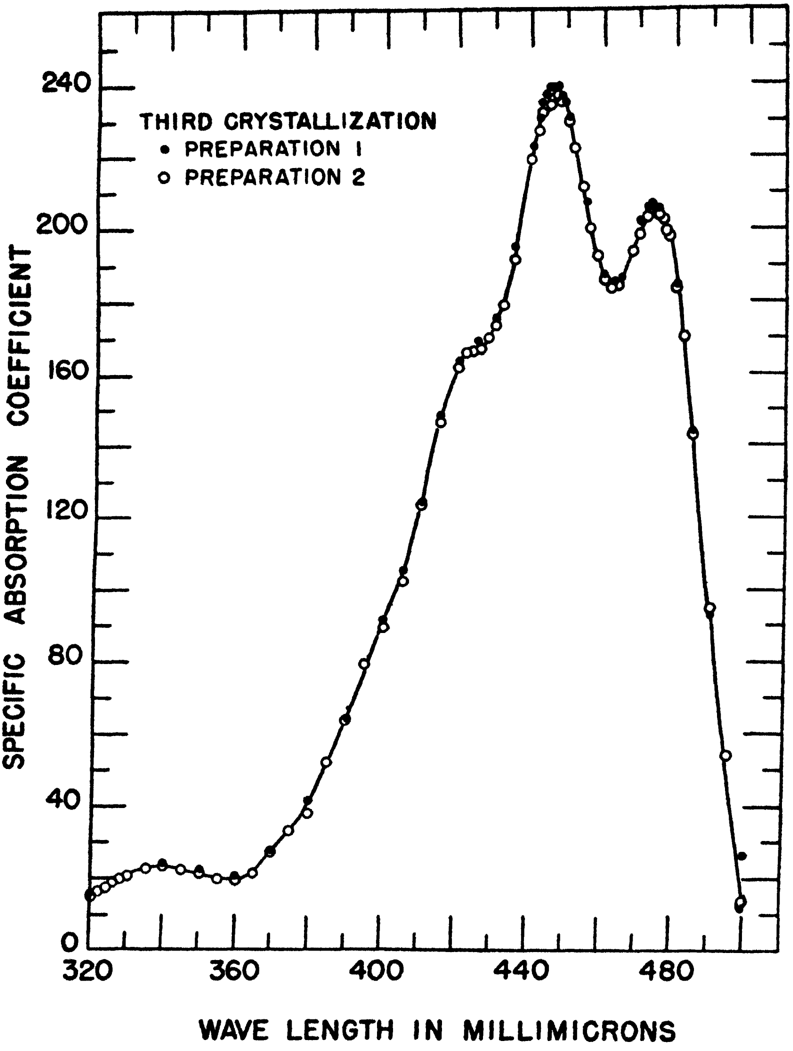

FIG. 3.—Absorption spectrum of neo-beta-carotene U in iso-octane.

tions agreed with each other within 2%. A second preparation prepared in the same way checked these values. Figure 3 presents the absorption spectra obtained for neo-beta-carotene U dissolved in iso-octane. Table 2 contains the specific absorption coefficients at the two maxima for this isomer.

Neo-beta-carotene B.—This isomer is much more unstable than is the neo-beta-carotene U, and thus is more difficult to prepare in the pure state. It is also more difficult to separate from the all-trans-beta-carotene and neo-beta-carotene E, which, are, respectively, just above and below it on the chromatographic column (16). In order to improve the separation of the zones during the development of the chromatogram, 1½% para-cresyl methyl ether was used as a developer instead of the 2% acetone. This material has been shown to increase the separation of the zones during the development of an isomerized beta-carotene solution on a hydrated lime column (2).

When the procedure described above for the preparation of crystalline neo-beta-carotene U was employed for the preparation of the B isomer, it was unsuccessful. Instead of crystals, only a viscous dark liquid resulted.

Since considerable difficulty was encountered in the preparation of neo-beta-carotene B, the procedure that was finally adopted is given in detail: ✓ A solution of purified beta-carotene in hexane (0.5 mg per ml) was isomerized as described above (see under carotene). This isomerized carotene was then poured on a No. VI hydrated lime column packed to a height of 28 cm. For best separation, not more than 40 mg total carotene could be added to each column. In this work, 4 columns were developed at the same time with 1½% para-cresyl methyl ether in hexane until the lower edge of the neo-beta-carotene E zone reached the bottom of the column. The development was then discontinued, the columns extruded, and the portions of the lime containing the neo-beta-carotene B zone were rapidly carved out and placed together on an 8-inch-diameter Büchner funnel. The carotene was eluted from the lime with the following eluents, respectively: 20% ethyl alcohol in hexane, 80% ethyl alcohol in hexane, and finally 100% ethyl alcohol. The reason for the succession of solvents was to elute the pigment with a minimum of hexane and not precipitate the carotene on the lime. About 1½ to 2 liters of eluent were required at this stage.

The eluate was washed in a continuous washing device to remove the alcohol, and dried. To concentrate the dilute carotene solution in a small volume for rechromatographing, the solution was adsorbed on magnesia-Dicalite (1 g adsorbent to 2.5 mg carotene) in a No. II column. The adsorbed carotene was eluted with 10% acetone in hexane, and the acetone removed by continuous washing. The use of a magnesia-Dicalite column permitted a liter of dilute carotene solution to be concentrated rapidly to 50 to 75 ml with little or no reisomerization. The partially purified neo-beta-carotene B solution in hexane was rechromatographed on a No. VI lime column. After three such chromatographic adsorptions on lime, a homogeneous band of neo-beta-carotene B free of the all-trans isomer was obtained. The isomer thus obtained was adsorbed on magnesia-Dicalite as described above, and 50 ml of a low-boiling fraction of petroleum ether (37–43°C.) was drawn through the column to remove the hexane and para-cresyl methyl ether which might have interfered with crystallization. The neo-beta-carotene B was eluted with reagent acetone, the solution was transferred to a 125-ml separatory funnel, and the acetone removed by continuous washing. The resulting carotene solution, containing about 20 to 30 mg of carotene in 2 ml of the low-boiling petroleum ether, was carefully filtered through an asbestos filter pad on a micro filter funnel directly into a conical centrifuge tube, and 8 ml of methyl alcohol were added. Crystals did not form at once, even when the liquid was cooled.

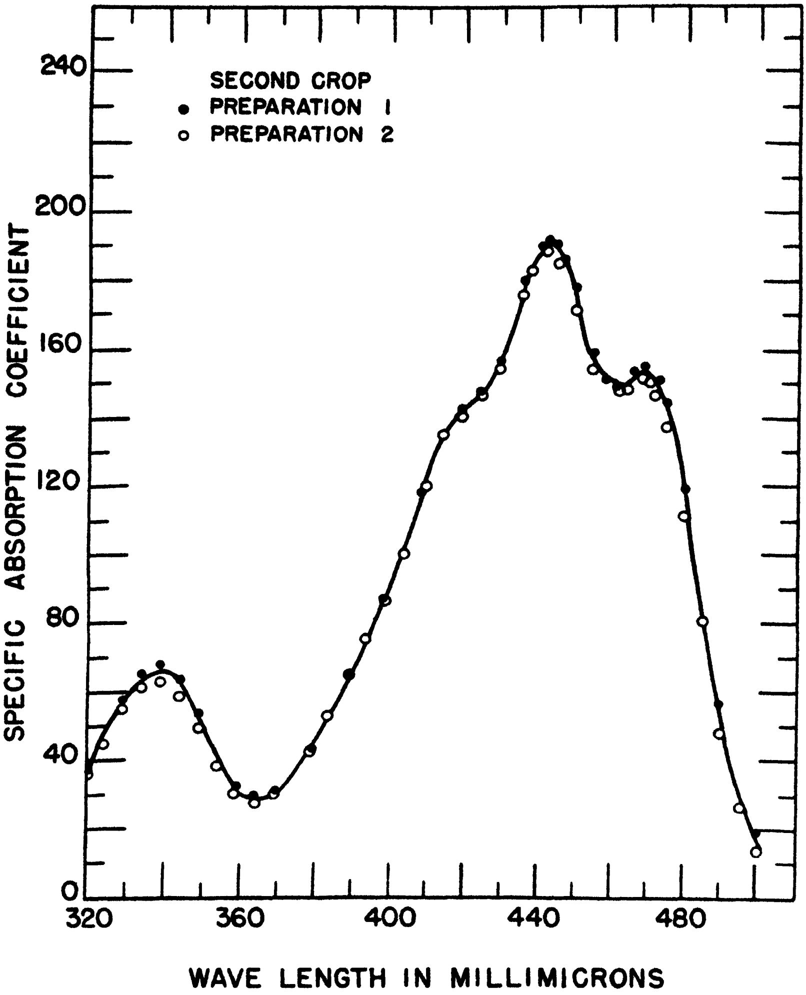

FIG. 4.—Absorption spectrum of neo-beta-carotene B in iso-octane.

However, after nitrogen (passed over hot, reduced copper to remove oxygen) had been bubbled through the liquid for several minutes, a crop of crystals in small spherulitic aggregates was formed. The crystals were removed by centrifugation, washed with redistilled methyl alcohol and dried under vacuum for one hour at room temperature. The mother liquor decanted from this first crop of crystals was stored for 2 hours at −30°F. under nitrogen, and a second crop of crystals was obtained. Again, the crystals were removed from the mother liquor by centrifuging, and dried for one hour. A third crop of crystals was obtained by storing the mother liquor overnight at −30°F. under CO_2. Chromatographic analysis of these crystalline preparations on hydrated lime showed only a very slight trace of all-trans-beta-carotene. The specific absorption coefficients for the second and third crops of crystals were almost identical, and were higher than that for the first crop. This entire preparative procedure was repeated. The absorption spectra for both preparations are given in Figure 4. Specific absorption coefficients at the wave length of maximum absorption are given in Table 2.

TABLE 3.—*The wave length of maximum absorption, and the specific absorption coefficient at this maximum, for all-trans-beta-carotene in various solvents*

SOLVENT	WAVE LENGTH mμ	SPECIFIC ABSORPTION COEFFICIENT
Iso-octane	451	251
Commercial hexane (65°–69°)[a]	451	251
Purified hexane B.P. 65°–67°	451	250
Petroleum ether B.P. 30°–60°[b]	449	253
Petroleum ether B.P. 30°–60°[b]	451	249
Petroleum ether B.P. 88°–99°[b]	453	247

[a] Phillips Petroleum Co.
[b] Skelly Oil Co.

DISCUSSION

Although all-trans-beta-carotene would seem to be readily purifiable by crystallization and chromatography, it has proved difficult for various investigators to obtain reproducible quantitative spectral absorption data even with related solvents (compare hexanes, Table 1). In order to evaluate the relative effect of solvent on the absorption coefficient, a single purified preparation of all-trans-beta-carotene was used. Portions of this material were dissolved in the various solvents, and data were obtained immediately with the same instrument. The data (Table 3) show that the different values reported in the literature may be attributed only partly to differences in solvent. However, since the specific solvent affects the absorption coefficient as well as the location of the absorption maxima, very consistent determinations by spectrophotometric measurements and published absorption coefficients can be obtained only if the same solvent is used in making the determination as was used for the constant. O'Connor and co-workers (14) discussed this problem and suggested iso-octane as a standard solvent. Iso-octane can be obtained in a very pure state, is readily available, and its cost is not excessive since it can be redistilled and reused without change in composition. Our results indicate that the absorption spectra of the various carotenes are very similar in iso-octane and hexane. This conclusion was reached also by Zscheile and co-workers for all-trans-alpha and all-trans-beta-carotene (24).

Some of the differences in the literature may have resulted from use of different instruments in making the spectrophotometric measurements. Instrumental differences are known to exist. To check our instrument, absorption coefficients were determined on a purified all-trans-beta-carotene preparation with two other instruments of the same make. The data thus obtained all agreed within 0.2 per cent. This agreement does not of course preclude the possibility that other instruments of different resolving power might give significantly different results.

Since no highly purified carotenes are commercially available, it is necessary for each laboratory to prepare its own carotene standards. Variations in spectral constants reported may be the result mainly of different methods of preparation, and it is here that improvements should be sought with the aim of developing uniform procedures. The values obtained in our laboratory for the specific absorption coefficients of all-trans-alpha- and all-trans-beta-carotene check closely with those reported by several other investigators (13, 19, 21). However, values several per cent higher have also been reported recently (5, 23, 24). It would seem reasonable to assume that the purer the preparation is, the higher the coefficient will be. It is difficult, however, to understand some of the extremely high values (Table 1; ref. 7, 12, 20).

Polgar and Zechmeister have described the preparation of crystalline neo-beta-carotene U (16), and have presented absorption constants for this isomer in hexane solution (23). No other quantitative data for this stereoisomer are available in the literature. In the present study the absorption spectrum of this isomer has been obtained in purified hexane, in iso-octane, and in two petroleum ethers (b.p. 63°–70°; 88°–98°, Skelly Oil Co.). Almost identical values were obtained in these solvents. However, our values are 5 to 6 per cent lower than those reported by Polgar and Zechmeister, and the value of the coefficient was not raised by repeated recrystallization of the carotene (Table 2).

Gillam and El Ridi (9) described the preparation of neo-beta-carotene B, which they termed pseudo-alpha-carotene. However, they did not obtain absorption coefficients for their preparation. Beadle and Zscheile (1) obtained quantitative absorption data for neo-beta-carotene B by an indirect method. They determined the absorption spectrum in hexane solution immediately after separating the isomer from the all-trans-beta-carotene by chromatography on an alumina column. The quantity of neo-beta-carotene B present in the hexane solution was then determined by evaporation of aliquots of the hexane solution to dryness and weighing the residual carotene. They explain that this procedure was used because of the great tendency for the stereoisomer to reisomerize back to the all-trans form during its isolation and crystallization. It is probable that any neo-beta-carotene E that might have been present in their original extract would have been included together with the neo-beta-carotene B, since it would appear as a zone immediately below the neo-beta-B on the developing column (16). The average specific absorption coefficient for this isomer obtained in this laboratory with crystalline preparations was about 8 per cent lower than that reported by Beadle and Zscheile (1). The slight amount of reisomerization during the preparative procedure would tend to raise the value. Our lower value, therefore, can not be accounted for on this basis.

The specific absorption coefficients of the various isomers reported here are in each case averages from at least two preparations, obtained immediately in most cases, and never more than 24 hours after preparation. All preparations except that of neo-beta-carotene B showed but a single homogeneous band when examined chromatographically on hydrated lime. The neo-beta-carotene B showed a second extremely faint band, which was attributed to all-trans-beta-carotene. The methods employed for the separation and crystallization of the neo-beta-carotenes B and U in the preparative procedure are laborious, but seem to be satisfactory from the standpoint of purity of the resulting material.

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SUMMARY

The following carotene isomers were isolated and purified by chromatographic and crystallization techniques: All-trans-alpha-carotene, all-trans-beta-carotene, neo-beta-carotene U, neo-beta-carotene B. The details of isolation and purification are described. The absorption spectrum of each was determined in iso-octane and in commercial hexane from 320 to 500 millimicrons by a photoelectric spectrophotometric method. The specific absorption values given are considered suitable as a basis of analysis for the individual isomers. The values thus obtained are compared with those obtained by other workers. Differences due to solvents are discussed.

LITERATURE CITED

(1) Beadle, B. W., and Zscheile, F. P., *J. Biol. Chem.*, **144**, 21 (1942).
(2) Bickoff, E. M., *Anal. Chem.*, **20**, 51 (1948).
(3) Deuel. H. J., Jr., Johnston, C., Sumner, E., Polgar, A., and Zechmeister, L., *Arch. Biochem.*, **5**, 107 (1944).

(4) DEUEL, H. J., JR., JOHNSTON, C., MESERVE, E. R., POLGAR, A., and ZECHMEISTER, L., *Arch. Biochem.*, 7, 247 (1945).
(5) DEVINE, J., HUNTER, R. F., and WILLIAMS, N. E., *Biochem. J.*, 39, 5 (1945).
(6) DUTTON, H. J., BAILEY, G. F., and KOHAKE, E., *Ind. Eng. Chem.*, 35, 1173 (1943).
(7) FREY, C. N., and STEELE, H. K.; reported by KEMMERER, A. R., *This Journal*, 25, 889 (1942).
(8) GILLAM, A. E., *Biochem. J.*, 29, 1831 (1935).
(9) GILLAM, A. E., and EL RIDI, M. S., *Biochem. J.*, 30, 1735 (1936).
(10) KEMMERER, A. R., and FRAPS, G. S., *J. Am. Chem. Soc.*, 66, 305 (1944).
(11) KEMMERER, A. R., *This Journal*, 28, 563 (1945).
(12) KUHN, R., (quoted by ZECHMEISTER, L., "Carotinoide," page 130, JULIUS SPRINGER, Berlin, 1934).
(13) MACKINNEY, G., and FRATZKE, W. E., *Anal. Chem.*, 19, 614 (1947).
(14) O'CONNOR, R. T., HEINZELMAN, D. C., and JEFFERSON, M. E., *Ind. Eng. Chem., Anal. Ed.*, 18, 557 (1946).
(15) PETERSON, W. J., HUGHES, J. S., and FREEMAN, H. F., *Ibid.*, 9, 71 (1937).
(16) POLGAR, A., and ZECHMEISTER, L., *J. Am. Chem. Soc.*, 64, 1856 (1942).
(17) SEIBERT, H. F. (reported by O'CONNOR, *et al.* (14).
(18) SHREWSBURY, C. L., KRAYBILL, H. R., and WITHROW, R. B., *Ind. Eng. Chem., Anal. Ed.*, 10, 253 (1938).
(19) SILKER, R. E., SCHRENK, W. G., and KING, H. H., *Ind. Eng. Chem., Anal. Ed.*, 16, 513 (1944).
(20) SMAKULA, A., *Zeitschr. angew. Chem.*, 47, 657 (1934).
(21) STRAIN, H. H., *J. Biol. Chem.*, 127, 191 (1939).
(22) ZECHMEISTER, L., and CHOLNOKY, "Principles and Practice of Chromatography," Figure 19, page 62, New York, John Wiley & Sons, Inc., 1941.
(23) ZECHMEISTER, L., and POLGAR, A., *J. Am. Chem. Soc.*, 65, 1522 (1943).
(24) ZSCHEILE, F. P., WHITE, J. W., JR., BEADLE, B. W., and ROACH, J. R., *Plant Physiol.*, 17, 331 (1942).

COMBINATION OF CATALYSTS IN THE DETERMINATION OF NITROGEN

III. MEAT PRODUCTS

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INTRODUCTION

The time required for the determination of nitrogen has been materially reduced since Kjeldahl (1) first proposed his original method. This reduction of time was first made possible by Wilfarth (2) with the introduction into the reaction mixture of a catalyst such as mercury or copper. Gunning (3) followed shortly with suggestions that potassium sulfate would increase the rate of digestion. Recently, the use of selenium has been advocated as a catalyst. Although Lauro (4) was the first to investigate the use of selenium, this substance has been combined with other catalysts by several investigators (5 –14, incl.). Of these workers, several (9, 11, 12) have found this element to reduce materially the amount of nitrogen obtained in the analysis. Sandstedt (14), also, has found with selenium some loss of nitrogen on long heating.

Folin and Wright (15) suggested the use of a mixture of phosphoric and sulfuric acids with potassium sulfate, a ten per cent solution of ferric chloride being used as a catalyst. Several other catalysts have been used, including phosphorus pentoxide and potassium permanganate (16), vanadium pentoxide (17), and perchloric acid (18, 19). These substances do not seem, however, to possess any great advantage over copper, mercury, and selenium.

Nearly all of the investigations in which selenium has been used for the determinations of nitrogen have been with cereal products. In this communication, there are reported the results obtained when selenium is used in combination with other catalysts in the determination of nitrogen in meat products. No experimental data are available on the efficiency of selenium when combined with copper, mercury, and strong hydrogen peroxide, outside of investigations (20, 21) reported from this laboratory.

METHODS AND PROCEDURE

The Gunning method, which has been adopted by the Association of Official and Agricultural Chemists (22), as an official method, was employed as the general basic procedure in the investigation. It is probable that none of the results reported in the tables represent the actual amount of nitrogen in the samples, since digestions were stopped immediately after the samples were clear.

Canned meat products and fresh meat were used. The canned meats were ground to a fine consistency, if not already in this condition. The samples were thoroughly mixed, and one-gram samples were weighed for analysis. The fresh meat samples were freed of all fat, ground, and dried for twelve to fifteen hours at 60°C. Each sample was then powdered in a mortar and dried for another twelve hours in a vacuum oven at 70°C. The percentages of nitrogen were calculated back to the original undried fresh meat after the amount of moisture present in each sample had been determined.

Ten grams of potassium sulfate and 20 cc of concentrated sulfuric acid were then added to each of the eight digestion flasks containing 1 gram samples of a given meat product with the following exceptions:

(1) In number (6), 4 g of potassium sulfate, 6 g of di-potassium phosphate, and 20 cc of sulfuric acid were added (23).

(2) In number (7), 15 cc of sulfuric acid and 5 cc of glacial phosphoric acid were added, the potassium sulfate being omitted.

The following catalysts then were added separately to one of the eight digestion flasks:

(1) Five-tenths g of mercuric oxide, with one cubic centimeter of 30 per cent hy-

TABLE 1.—*Determination of nitrogen in canned meat products*

CATALYST	POTTED MEAT		VIENNA SAUSAGE		DEVILED HAM		DEVILED MEAT		MEAT SPREAD	
	MINUTES FOR CLEARING	NITROGEN	MINUTES FOR CLEARING	NITROGEN	MINUTES FOR CLEARING	NITROGEN	MINUTES FOR CLEARING	NITROGEN	MINUTES FOR CLEARING	NITROGEN
		per cent		*per cent*		*per cent*		*per cent*		*per cent*
(1) $HgO+H_2O_2$	20	3.48	21	1.98	25	2.41	22	2.70	25	3.11
(2) $Se+H_2O_2$	20	3.42	13	2.07	24	2.45	20	2.70	22	3.02
(3) $CuSO_4+H_2O_2$	25	3.38	25	1.99	32	2.35	26	2.70	36	2.97
(4) $HgO+Se+CuSO_4$	16	3.44	13	2.00	21	2.30	16	2.73	15	3.12
(5) $HgO+Se+CuSO_4+H_2O_2$	15	3.42	12	1.96	18	2.40	15	2.72	16	3.05
(6) $HgO+Se+CuSO_4+H_2O_2+K_2HPO_4$	11	3.45	8	2.08	16	2.49	14	2.73	13	3.09
(7) $Se+CuSO_4+H_3PO_4$	19	3.43	19	2.00	24	2.43	16	2.70	19	2.96
(8) Control	90	3.45	75	1.98	90	2.41	115	2.71	120	3.07
Average		3.43		2.00		2.40		2.71		3.04

TABLE 2.—*Determination of nitrogen in fresh meats*

CATALYST	CHICKEN GIZZARD		CHICKEN		VEAL		BEEF		LAMB		PORK	
	MINUTES FOR CLEARING	NITROGEN	MINUTES FOR CLEARING	NITROGEN	MINUTES FOR CLEARING	NITROGEN	MINUTES FOR CLEARING	NITROGEN	MINUTES FOR CLEARING	NITROGEN	MINUTES FOR CLEARING	NITROGEN
		per cent		*per cent*		*per cent*		*per cent*		*per cent*		*per cent*
(1) $HgO+H_2O_2$	24	3.35	24	3.06	25	3.65	25	3.26	21	3.53	23	3.98
(2) $Se+H_2O_2$	24	3.29	22	3.07	24	3.62	24	3.25	22	3.43	25	3.95
(3) $CuSO_4+H_2O_2$	28	3.30	27	3.03	27	3.56	28	3.19	25	3.45	25	3.92
(4) $HgO+Se+CuSO_4$	14	3.32	13	3.06	14	3.62	16	3.26	15	3.48	15	3.97
(5) $HgO+Se+CuSO_4$ $+H_2O_2$	14	3.33	12	3.07	13	3.58	15	3.25	13	3.48	14	3.97
(6) $HgO+Se+CuSO_4$ $+H_2O_2+K_2HPO_4$	12	3.32	12	3.07	12	3.63	14	3.25	13	3.46	12	3.93
(7) $Se+CuSO_4+H_3PO_4$	23	3.30	20	3.07	15	3.60	17	3.19	14	3.42	16	3.92
(8) Control	90	3.32	145	3.07	100	3.61	105	3.25	120	3.46	120	3.95
Average		3.31		3.06		3.61		3.24		3.46		3.94

drogen peroxide added at the beginning of the digestion, and one cubic centimeter every five minutes thereafter, until the digestion mixture cleared as recommended by Poe and Dewey (24).

(2) Two-tenths g of selenium, with one cubic centimeter of hydrogen peroxide added every five minutes as in (1).

(3) One gram copper sulfate with one cubic centimeter of hydrogen peroxide added as in (1).

(4) Three-tenths g mercuric oxide, one-tenth g selenium, and five-tenths g copper sulfate.

(5) Three-tenths g mercuric oxide, one-tenth g selenium, five-tenths g copper sulfate and hydrogen peroxide added as in (1).

(6) The same catalysts as in (5) plus di-potassium hydrogen phosphate.

(7) One-tenth g selenium, and five-tenths g copper sulfate, plus phosphoric acid.

(8) A control with no catalysts added.

TABLE 3.—*Average results for clearing time and percentage of nitrogen of meat products*

CATALYST	CLEARING TIME—MINUTES			PER CENT NITROGEN	
	CANNED MEAT	FRESH MEAT	AVERAGE	CANNED MEAT	FRESH MEAT
(1) $HgO+H_2O_2$	22.6	23.7	23.2	2.74	3.47
(2) $Se+H_2O_2$	19.8	23.5	21.7	2.73	3.44
(3) $CuSO_4+H_2O_2$	28.8	26.7	27.8	2.68	3.41
(4) $HgO+Se+CuSO_4$	16.2	14.5	15.4	2.72	3.45
(5) $HgO+Se+CuSO_4+H_2O_2$	15.2	13.5	14.4	2.71	3.45
(6) $HgO+Se+CuSO_4$ $+H_2O_2+K_2HPO_4$	12.4	12.5	12.5	2.77	3.44
(7) $Se+CuSO_4+H_3PO_4$	19.4	17.5	18.5	2.70	3.42
(8) Control	98.0	113.3	105.7	2.72	3.44

The amounts of nitrogen in different meat samples were determined under each of the eight conditions listed above. A blank determination was run on all reagents (1) to (8) inclusive. The percentages of nitrogen obtained and the time of clearing for the canned and fresh meats are listed in Tables 1 and 2. The average clearing times and percentages of nitrogen for all samples by each modification are given in Table 3.

The addition of potassium hydrogen phosphate in combination number 6 seemed to reduce the clearing time to a marked degree. An attempt was made to find the combination of potassium sulfate and di-potassium phosphate which would give the most rapid clearing of the digestion mixture. The catalysts used were three-tenths gram mercuric oxide, one-tenth gram selenium, five-tenths gram copper sulfate, with one cubic centimeter of 30 per cent hydrogen peroxide being added every five minutes. The amounts of potassium sulfate and di-potassium phosphate used and the corresponding times required for clearing are given in Table 4.

TABLE 4.—*Results obtained with varying amounts of potassium sulfate and di-potassium hydrogen phosphate**

DETERMINATION OF NITROGEN IN DRIED BEEF			
GRAMS K_2SO_4	GRAMS K_2HPO_4	MINUTES FOR CLEARING	PER CENT NITROGEN
10	0	16	3.19
8	2	17	3.26
6	4	16	3.22
5	5	14	3.15
4	6	13	3.21
4	10	14	3.20
2	8	13	3.25
10	6	18	3.18
Average			3.21

* Catalysts used were HgO, Se, $CuSO_4$, and H_2O_2

DISCUSSION

A study of the tables shows a sharp decrease in clearing time when the three catalysts—mercuric oxide, selenium, and copper sulfate—are combined, as compared with the times when these catalysts were used independently. The addition of hydrogen peroxide to the preceding combination reduced the time still further, but probably not enough to justify the additional trouble and expense.

When, however, part of the potassium sulfate is replaced by di-potassium phosphate, with a retention of the same combination of catalysts—mercuric oxide, selenium, copper sulfate, and hydrogen peroxide—the clearing time is reduced considerably. In spite of the more rapid clearing time, the accuracy of the nitrogen determination does not seem to be seriously impaired.

The combination of copper sulfate and hydrogen peroxide, as shown by the tables, shows the greatest time for clearing and consistently gives low results for nitrogen content when the determination of nitrogen was made after the first clearing. This condition is covered by instruction in the official A.O.A.C. procedures (22) that mere clearing time is insufficient for complete digestion.

The combination of selenium and copper sulfate with phosphoric and sulfuric acids did not produce so satisfactory results as did the combination of selenium, copper sulfate, and mercuric oxide with potassium hydrogen phosphate and sulfuric acid.

CONCLUSIONS

1. Mercuric oxide, selenium, and copper sulfate, when used separately

with hydrogen peroxide, act about equally effectively in reducing the time necessary for clearing.

2. Copper sulfate used in combination with hydrogen peroxide gave slightly lower yields of nitrogen when determined at the time of clearing than were obtained with other catalysts.

3. A combination of mercuric oxide, selenium, and copper sulfate reduces the clearing time to approximately 60 per cent of the time required when either of these catalysts is used alone with hydrogen peroxide. Hydrogen peroxide added to the above mentioned combination has little effect in hastening the reaction further.

4. The addition of di-potassium hydrogen phosphate to the combination of mercuric oxide, selenium, copper sulfate, and hydrogen peroxide causes a reduction in clearing time of about 15 per cent over the combination without the potassium hydrogen phosphate.

REFERENCES

(1) KJELDAHL, J., *Zeit. Anal. Chem.*, **22**, 366 (1883).
(2) WILFARTH H., *Chem. Zentr.*, (3), **16**, 17, 113 (1885).
(3) GUNNING, J. W., *Zeit. Anal. Chem.*, **28**, 188 (1889).
(4) LAURO, M. F., *Ind. Eng. Chem., Anal. Ed.*, **3**, 401 (1931).
(5) RICH, C. E., *J. Cereal Chem.*, **9**, 118 (1932).
(6) MESSMAN, H. C., *Ibid.*, **9**, 357 (1932).
(7) HITCHCOCK, D. I., and BELDEN, R. C., *Ind. Eng. Chem., Anal. Ed.*, **5**, 402 (1933).
(8) OSBORN, R. A., and KRASNITZ, A., *This Journal*, **16**, 110 (1933).
(9) DAVIS, C. F., and WISE, K., *J. Cereal Chem.*, **10**, 488 (1933).
(10) TAYLOR, L. V., JR., *Ind. Eng. Chem., Anal. Ed.*, **5**, 263 (1933).
(11) OSBORN, R. A., and KRASNITZ, A., *This Journal*, **17**, 339 (1934).
(12) SNIDER, S. R., and COLEMAN, D. A., *J. Cereal Chem.*, **11**, 414 (1934).
(13) CAMPBELL, W. R., and HANNA, M. I., *J. Biol. Chem.*, **119**, 1 (1937).
(14) SANDSTEDT, R. M., *J. Cereal Chem.*, **9**, 156 (1932).
(15) FOLIN, O., and WRIGHT, L. E., *J. Biol. Chem.*, **38**, 461 (1919).
(16) FORD, J. S., TAIT, A., FLETCHER, L., SPIERS, J., and MITCHELL, W. J., *J. Inst. Brewing*, **39**, 472 (1933); *Analyst*, **58**, 618 (1933).
(17) PARRI, W., *Giron. Farm. Chem.*, **71**, 253 (1923).
(18) MEARS, B., and HUSSEY, R. E., *Ind. Eng. Chem.*, **13**, 1054 (1921).
(19) BIMMERMAN, P. H., and FRANK, W. L., *J. Amer. Assoc. Cereal Chem.*, **8**, 49 (1923). See *Chem. Abstracts*, **17**, 2156 (1923).
(20) POE, C. F., and NALDER, MARGARET, *Ind. Eng. Chem., Anal. Ed.*, **7**, 189 (1935).
(21) POE, C. F., and SCHAFER, R. R., *J. Dairy Science*, **18**, 733 (1935).
(22) *Methods of Analysis, A.O.A.C.*, Sixth Ed., Washington (1945).
(23) GERRITZ, H. W., and ST. JOHN, J. L., *Ind. Eng. Chem., Anal. Ed.*, **7**, 380 (1935).
(24) POE, C. F., and DEWEY, B. T., *J. Amer. Pharm. Assoc.*, **25**, 419 (1936).

ANALYSIS OF SULFADIAZINE-SULFATHIAZOLE MIXTURES

A SPECTROPHOTOMETRIC METHOD

By DANIEL BANES (U. S. Food and Drug Administration, Federal Security Agency, Chicago, Ill.)*

Increasing numbers of preparations containing a mixture of sulfonamides have appeared in recent years. Since official methods of assay for the individual compounds have been restricted largely to nitrite titrations of the active amino group common to all of them, it has become necessary to devise new procedures for the control analysis of specific constituents. The sulfonamides absorb ultra-violet light between 220 and 300 mμ, and their characteristic spectra differ, depending upon the amide substituent. Mixtures may therefore be analyzed by optical density measurements under varied conditions of absorption.

A simple, rapid spectrophotometric method can be designed for the analysis of preparations containing sulfadiazine and sulfathiazole. Sulfadiazine in strongly acid solution shows a peak absorption at 239 mμ, the optical density diminishing from that point to about 275 mμ, and flattening to a plateau from 275 to 300 mμ. Sulfathiazole yields a more symmetrical curve with a well-defined maximum near 278 mμ and a minimum at 240 mμ. (See the accompanying graph.)

By "two-color" analysis, measuring the optical densities of standards and unknown solutions in 1 cm cells at 239 and 280 mμ, a set of two equations in two unknowns can be established for the estimation of sulfadiazine and sulfathiazole. (Both compounds obey Beer's Law at the two wave-lengths.) Let

K_1 = extinction coefficient for sulfadiazine at 239 mμ
K_2 = extinction coefficient for sulfathiazole at 239 mμ
K_3 = extinction coefficient for sulfadiazine at 280 mμ
K_4 = extinction coefficient for sulfathiazole at 280 mμ
C_N = concentration of sulfadiazine as mg/100 ml HCl (1+3)
C_L = concentration of sulfathiazole as mg/100 ml HCl (1+3)
E_{239} = optical density of the unknown solution at 239 mμ
E_{280} = optical density of the unknown solution at 280 mμ.

Then, since the optical density of a mixture is the sum of the constituent densities,

$$E_{239} \times 1000 = K_1C_N + K_2C_L \qquad (1a)$$

$$E_{280} \times 1000 = K_3C_N + K_4C_L \qquad (1b)$$

These equations can be solved explicitly for C_N and C_L as functions of E_{239} and E_{280}:

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$$C_N = \frac{1000(K_4\,E_{239} - K_2\,E_{280})}{K_1K_4 - K_2K_3} \tag{2a}$$

$$C_L = \frac{1000(K_1\,E_{280} - K_3\,E_{239})}{K_1K_4 - K_2K_3} \tag{2b}$$

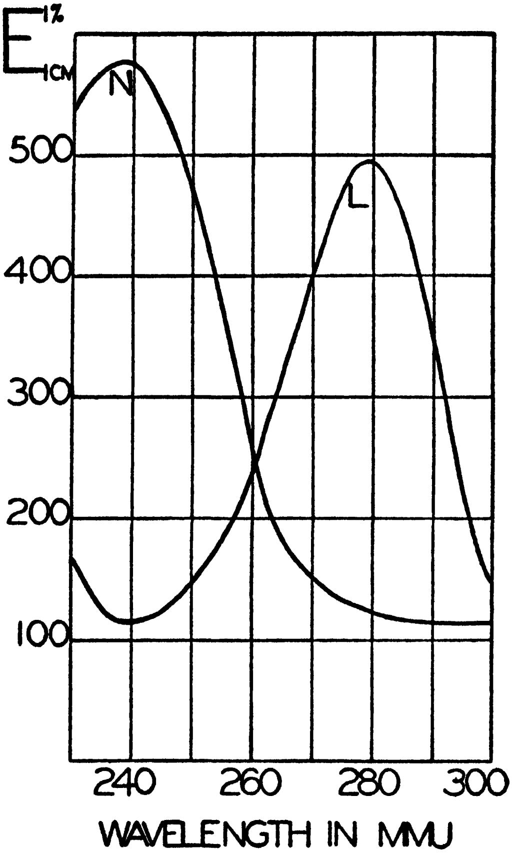

FIG. 1.—Absorption Spectra of Sulfonamides in 3N HCl.
N = Sulfadiazine
L = Sulfathiazole

PROPOSED SPECTROPHOTOMETRIC METHOD

Shake a convenient representative sample containing sulfadiazine and sulfathiazole with 200 ml HCl (1+3) in a 500 ml volumetric flask until the sulfonamides are completely dissolved. (For ointments transfer the sample to a 125-ml separatory funnel, dissolve in 50 ml of ethyl ether, and shake out with several 50 ml portions of HCl (1+3), collecting the aqueous layers in a 500 ml volumetric flask.) Dilute to the mark with the acid soln, mix thoroly and filter thru a dry folded filter paper if necessary. Transfer an aliquot containing 1–2 mg total sulfonamides to a 100 ml volumetric flask, and dilute to volume with HCl (1+3). Determine the optical densities of this solution at 239 and 280 mμ in the ultra-violet spectrophotometer, with distilled water as reference cell.

At the same time, using the same HCl (1+3) as diluent, prepare a soln containing 1.5 mg sulfadiazine of known purity per 100 ml and a soln containing 1.5 mg sulfathiazole of known purity per 100 ml. Measure the optical densities of these standards, and of the dilute HCl as correction blank, together with those of the unknown under identical conditions of wave length, slit-width, and cell thickness. From data on the standards compute the extinction coefficients and calculate the concentrations of sulfadiazine and sulfathiazole in the unknown soln from equations (2a) and (2b).

EXPERIMENTAL

Data obtained by the use of known 1.5 mg sulfonamide solutions yielded the following numerical values:

$K_1=555$; $K_2=106$; $K_3=103$; $K_4=469$. Substitution of these values in equations (2) gave:

$$C_N = 1.881\ E_{239} - 0.425\ E_{280} \tag{3a}$$

$$C_L = 2.225\ E_{280} - 0.413\ E_{239} \tag{3b}$$

Several samples containing varying proportions of sulfadiazine and sulfathiazole were analyzed according to the proposed method, using equations (3a) and (3b). Results of analysis are shown in Table 1. A commercial sample of tablets also assayed spectrophotometrically yielded the following results: Sulfadiazine, Declared 0.25 gm/tablet; Found 0.252 gm/tablet. Sulfathiazole, Declared 0.25 gm/tablet; Found 0.247 gm/tablet.

TABLE 1.—*Sulfadiazine-sulfathiazole mixtures*

PUT IN		RECOVERED			
SULFADIAZINE	SULFATHIAZOLE	SULFADIAZINE		SULFATHIAZOLE	
mg	*mg*	*mg*	*per cent*	*mg*	*per cent*
0.00	2.00	−0.011	—	2.021	101.0
0.25	1.75	0.248	99.3	1.764	100.8
0.50	1.50	0.495	99.1	1.519	101.3
0.75	1.25	0.739	98.2	1.274	101.9
1.00	1.00	0.995	99.5	1.026	102.6
1.25	0.75	1.234	98.7	0.776	103.4
1.50	0.50	1.506	100.4	0.524	104.8
1.75	0.25	1.775	101.4	0.256	102.5
2.00	0.00	2.043	102.1	0.003	—

CONCLUSIONS

A simple rapid method has been proposed for the analysis of mixtures containing sulfadiazine and sulfathiazole. The method is suitable for the assay of pharmaceuticals containing these sulfonamides.

POLYMORPHIC MODIFICATIONS OF SULFANILAMIDE

By MORRIS L. YAKOWITZ (U. S. Food and Drug Administration, San Francisco, Calif.)

Van Zyp (1) observed that sulfanilamide appears in several crystalline forms when crystallized from a drop of water and examined under the microscope.

Watanabe (2) made on X-ray diffraction study of sulfanilamide crystallized from various solvents and determined that sulfanilamide can crystallize in at least three polymorphic modifications, one of which is orthorhombic and the other two monoclinic.

Prien and Frondel (3) made a study of sulfanilamide deposited from urine and reported that it crystallizes in the orthorhombic system and has the refractive indexes, $\alpha = 1.550$, $\beta = 1.622$, and $\gamma = 1.80$.

Keenan (4), in his report on the refractive indexes of substances in the USP XII, gave the refractive indexes of sulfanilamide as $\alpha = 1.570$, $n_i = 1.677$, $\gamma = >1.733$.

An examination of sulfanilamide crystallized from various solvents has confirmed Watanabe's discovery that sulfanilamide is polymorphic with at least three phases. The refractive indexes of the several forms with their probable crystal system are as follows:

DESIGNATION	LOWEST R.I. α	INTERMEDIATE R.I. β	HIGHEST R.I. γ	CRYSTAL SYSTEM
Modification I	1.547	1.622	>1.733	Orthorhombic
Modification II	1.558	1.675	>1.733	Monoclinic
Modification III	1.500	1.673	>1.733	Monoclinic

Modifications I and II can be obtained from water, ethyl alcohol, acetone, and n-propyl alcohol. Under some conditions of crystallization, the crystals formed from these solvents may be largely Modification I, while under different conditions of crystallization, Modification II will predominate.

Modification I tends to form as elongated laths giving a centered interference figure on the flattened side with β parallel to the long direction and α crosswise.

Modification II tends to form as equant blocks with an uncentered interference figure when lying in their preferred position. Such crystals give β and another intermediate R.I.

When either Modification I or II is heated, the crystals transform into Modification III at about 110°C. to 130C°. Modification III is the only stable phase above about 130°C. When large crystals of I or II are heated to about 130°C., they become chalky and fissured when they

transform into III. Small, thin crystals of I or II transform smoothly and completely into III when heated and exhibit the refractive indexes of III while retaining the outward appearance of I or II.

III is the phase which forms when molten sulfanilamide is cooled. III may be obtained from toluene solution in the form of elongated lath-like crystals.

REFERENCES

(1) C. VAN ZYP, *Pharm. Weekblad*, **75**, 585 (1938).
(2) A. WATANABE, *Naturwiss enschaften*, **29**, 116 (1941) thru C.A. **36**, 695 (1942).
(3) E. L. PRIEN and C. FRONDEL, *J.Urol.*, **46**, 748 (1941).
(4) G. L. KEENAN, *This Journal*, **27**, 153 (1944).

DETERMINATION OF THE NITROGEN CONTENT OF PROTEINS AND AMINO ACIDS CONTAINING REFRACTORY NITROGEN BY MICRO- AND MACROKJELDAHL METHODS

By LAWRENCE M. WHITE, GERALDINE E. SECOR, and MARION D. CARTER LONG (Western Regional Research Laboratory,[1] Albany, Calif.)

The recent excellent reviews by Kirk (1) and Dunn and Rockland (2) forcibly called attention to the difficulties involved in the use of the Kjeldahl method to determine the total nitrogen content of proteins and amino acids containing refractory nitrogen. Of the various difficulties encountered in this method, analysts have probably given the most attention to the composition of the catalyst and the length of the digestion period. Chibnall, Rees, and Williams (3), Jonnard (4), Dunn and Rockland (2), and others have stressed the need for 6- to 8-hour digestion periods for proteins and amino acids. Many workers have used digestion catalysts that have been shown to be inferior to mercury in promoting conversion of the organic nitrogen to ammonia, and others, finding that tyrosine (5, 6) and tryptophan (5) were not completely decomposed with the usual digestion times, have assumed that proteins would also require extended digestion.

Success in determining the total nitrogen in tryptophan and in gramicidin (a polypeptide containing about 40% tryptophan) by minor modifications (7) of the A.O.A.C tentative microkjeldahl method (8) requiring a total digestion time of approximately 80 minutes, prompted a study of the recovery of nitrogen from the other amino acids containing refractory nitrogen, and from typical proteins, by the official macro- and the tentative microkjeldahl methods. Accordingly, lysine (2, 3, 5, 9, 10), histidine (3, 10), tryptophan (5, 9), tyrosine (5, 6), and a group of crystallized or otherwise purified proteins selected for their high content of these amino acids, were analyzed by these methods. The data presented below show that the precision and accuracy of the methods are satisfactory for these materials.

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[1] Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

EXPERIMENTAL

Methods:

Microkjeldahl.—The tentative microkjeldahl method (8) as modified by White and Secor (7) was used. In certain cases the digestion time was increased from the standard 80 minutes to 110 or 360 minutes. A Scientific Glass Apparatus Co.[2] No. M3074 microkjeldahl still was used.

Macrokjeldahl.—Samples containing 15 to 25 mg nitrogen were digested according to the Kjeldahl-Gunning-Arnold official method (mercuric oxide catalyst) (11) on an electrically heated rack for a period of two hours after becoming clear and colorless. The ammonia was distilled into 50 ml of 5% boric acid and titrated with 0.07 *N* sulfuric acid using methyl orange-xylene cyanole indicator (Eastman No. 2216).[2]

Equilibration and Moisture.—All samples were equilibrated several days at approximately 27° and 42% relative humidity, and subsequent weighings were made under these conditions. For moisture determinations, 50-mg portions of the equilibrated materials were heated 16 hours at 102±2° in air. Great care was taken to prevent the reabsorption of water by the dried sample. All results presented in the tables have been corrected for the moisture contents of the samples.

Materials:

Amino Acids.—Merck[2] L-lysine monohydrochloride, DL-lysine monohydrochloride, DL-tryptophan, L-histidine monohydrochloride monohydrate, and L-tyrosine were used without purification. As a basis for comparing the various times and methods of digestion, the amino acids were assumed to be pure.

Proteins.—The protein samples used were obtained as indicated under "Acknowledgments" and may be characterized as follows:

Bovine plasma albumin, crystalline, Armour[2] lot 46.

Casein I, prepared and reprecipitated according to Van Slyke and Baker (12).

Casein II, purified, nitrogen content 15.61% by the method of Chibnall *et al.* (3).

Chymotrypsinogen I, crystalline. A commercial preparation was recrystallized 7 times according to the procedure of Kunitz and Northrop (13) and dialyzed.

Chymotrypsinogen II, crystalline, dialyzed and crystallized from dilute alcohol.

β-Lactoglobulin, crystalline, recrystallized 4 times according to Palmer (14).

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[2] The mention of this product does not imply that it is endorsed or recommended by the Department of Agriculture over others of a similar nature not mentioned.

Lysozyme I, crystalline, crystallized and dialyzed according to Alderton and Fevold (15).

Lysozyme II, crystalline, crystallized 3 times at *p*H 4 by the method of Alderton and Fevold (15) and electrodialyzed.

Ovalbumin, crystalline, prepared and recrystallized 3 times by the method of Kekwick and Cannan (16).

Bovine methemoglobin, prepared according to Anson (17), nitrogen content 16.66% by the method of Chibnall *et al.* (3).

TABLE 1.—*Nitrogen recovery from amino acids*

AMINO ACID	N CONTENT (THEORETICAL) PER CENT	DIGESTION[1] TIME, MINUTES	NITROGEN RECOVERED[2] BY			
			MICRO METHOD[3]		MACRO METHOD[4]	
			PER CENT	PER CENT OF THEORETICAL	PER CENT	PER CENT OF THEORETICAL
DL-lysine HCl	15.34	80	15.20	99.09	15.19	99.02
		110	15.26	99 48		
		360	15.38	100.26		
L-lysine · HCl	15.34	80	15.24	99.35	15.25	99.41
		110	15.40	100.39		
		360	15.41	100.46		
DL-tryptophan	13.72	80	13.52	98.54	13.71	99.92
		110	13.66	99.56		
		360	13.70	99.85		
L-histidine $HCl \cdot H_2O$	20.05	80	20.12	100.35	20.06	100.05
		110	20.12	100.35		
		360	20.14	100.45		
L-tyrosine	7.73	80	7.74	100.13	7.80	100.77
		110	—	—		
		360	7.74	100.13		

[1] Macro digestions were continued 120 minutes after the liquid cleared.

[2] Recoveries are the averages of 3 to 11 unselected replications of the stated conditions.

[3] Individual results differed from the means of their respective groups by an average of 0.025% *N* and 87% of them differed by 0 04% or less

[4] Individual results differed from the means of their respective groups by an average of 0.067% *N* and 81% of them differed by 0 11% or less.

DISCUSSION OF RESULTS

Table 1 shows that lysine and tryptophan yielded 98.5 to 99.9 per cent of their nitrogen by the 80-minute micro and the macro digestions. Recovery of nitrogen from these acids by the micro method increased with time, but recoveries from histidine and tyrosine were complete after the 80-minute micro and the macro digestions.

Chibnal *et al.* (3) showed that recovery of nitrogen from lysine was more difficult than it was from β-lactoglobulin, a protein rich in lysine.

This is to be expected, since proteins rarely contain more than 10 to 12 per cent of lysine; therefore, the maximum amount of nitrogen that would not be recovered by methods used in this study would approximate only 0.02 to 0.04 per cent, an amount which is within the experimental error of the method. Likewise, proteins rich in tryptophan would appear to give quantitative recoveries more readily than does the pure amino acid.

TABLE 2.—*Nitrogen content of various proteins*

PROTEIN	MOISTURE	DIGESTION[1] TIME	NITROGEN CONTENT (MOISTURE-FREE BASIS)		
			MICRO METHOD	MACRO METHOD	LITERATURE OR OTHER ANALYSIS
	Per Cent	*Minutes*	*Per Cent*	*Per Cent*	*Per Cent*
Bovine met-hemoglobin	9.11	80	16.73 (16.71, 16.74, 16.75)		16.66[2]
		120		—	
		360	16.73 (16.71, 16.73, 16.75)		
Bovine plasma Albumin	8.58	80	16.08 (16.07, 16.09)		16.07 (*18*)
		120		16.02 (15.96, 16.08)	
		360	15.96 (15.93, 15.98)		
Casein I	7.65	80	15.44 (15.43, 15.44)		15.61 (*3*)
		120		15.39 (15.32, 15.45)	
		360	15.41 (15.38, 15.43)		
Casein II	8.59	80	15.72 (15.71, 15.73)		15.61[2]
		120		15.68 (15.55, 15.81)	
		360	15.74 (15.73, 15.74)		
Chymotrypsinogen I	8.78	80	16.47 (16.44, 16.49)		15.87 (*19*)
		120		16.38 (16.37, 16.38)	
		360	16.48 (16.47, 16.49)		
Chymotrypsinogen II	9.35	80	16.42 (16.40, 16.43, 16.44)		15.87 (*19*)
		120		16.49 (16.47, 16.50)	
		360	16.44 (16.41, 16.45, 16.45)		
β-Lactoglobulin	9.20	80	15.66 (15.64, 15.68)		15.60 ± 02 (*20*)
		120		15.70 (15.66, 15.73)	
		360	15.65 (15.65, 15.65)		
Lysozyme I	9.75	80	18.60 (18.55, 18.64)		
		120		18.50 (18.49, 18.50)	—
		360	18.56 (18.55, 18.56)		
Lysozyme II	10.04	80	18.34 (18.31, 18.35, 18.37)		
		120		18.34 (18.32, 18.36)	—
		360	18.35 (18.34, 18.35, 18.36)		
Ovalbumin	8.06	80	15.72 (15.71, 15.73)		15.68 (*3*)
		120		15.61 (15.59, 15.62)	
		360	15.71 (15.68, 15.73)		

[1] Macro digestions continued 120 minutes after liquid cleared.

[2] Analysis of this sample by the method of Chibnall *et al.* (3).

Table 2 shows that none of the proteins studied had a higher apparent nitrogen content after prolonged digestion, the 80-minute procedure being sufficient in all cases to give values in agreement (except for chymotrypsinogen) with the generally accepted values in the literature or with those determined by Chibnall's method. The micro and macro methods employed in this study gave results that are in excellent agreement.

Chymotrypsinogen has been reported by Brand and Kassell (19) to contain 15.87% *N* (moisture-free basis) and 16.18% *N* after correction for the 1.94% sulfate content of the sample. Analysis showed that chymotrypsinogen I has only 0.04 per cent of ash (constant weight at 550° with no additives) and 0.75 per cent of sulfate (moisture-free basis). Its nitrogen content (moisture- and sulfate-free basis) is 16.59 per cent. Chymotrypsinogen I, crystallized 8 times and exhaustively dialyzed (*cf.* low sulfate content) and the chymotrypsinogen II prepared by Dr. Kunitz have the same apparent nitrogen content. It is therefore believed that the new value (16.59%) reported here more closely approximates the true nitrogen content of chymotrypsinogen. The difference between our result and that reported previously may lie in the difficulties encountered in determining the moisture content of proteins. Chibnall *et al.* (3) have discussed this point at length.

To determine whether hydrolysis of the protein before digestion would affect the recovery of nitrogen, 5–7 mg samples of bovine plasma albumin, casein I, β-lactoglobulin, and lysozyme I were heated 4 hours at 95 to 100° in open microkjeldahl flasks with 2 ml of 27 *N* sulfuric acid. Recoveries after 80- and 110-minute digestions (counting the time from the appearance of sulfuric fumes) were identical within experimental error, and the results were in agreement with those obtained on the unhydrolyzed samples.

It can be concluded from this study that the 6–8 hour digestions recommended by many other workers are not needed for the complete recovery of amino and heterocyclic nitrogen from amino acids and proteins. Therefore the time-saving methods used in this work may be safely employed for the determination of total nitrogen in proteins and amino acids. However, it should not be considered that these methods suffice for all nitrogenous compounds. For example, Ogg, Brand, and Willits (21) and Shirley and Becker (22), working on the micro and macro scales, respectively, and using mercury-selenium catalysts, have shown that digestion periods up to four hours are necessary to obtain complete recovery of nitrogen from certain heterocyclic nitrogenous compounds. We have confirmed these findings in our laboratory.

ACKNOWLEDGMENT

This study would not have been possible without the generous cooperation and counsel of those who furnished samples of pure proteins. We thank Moses Kunitz for the chymotrypsinogen II, E. F. Jansen for the chymotrypsinogen I and the β-lactoglobulin, G. Alderton for the lysozymes, H. S. Olcott and D. K. Mecham for the casein I and the ovalbumin,

Leo Kline for the bovine methemoglobin, and R. C. Warner for the casein II.

SUMMARY AND CONCLUSIONS

Amino acids containing refractory nitrogen and a group of purified proteins rich in these acids were analyzed by the tentative microkjeldahl method employing mercuric oxide catalyst with an 80-minute digestion and also by the Official Kjeldahl-Gunning-Arnold macro method. Recovery of nitrogen from the amino acids was at least 98.5% complete by both methods. Results obtained for the proteins were concordant and, except for chymotrypsinogen, were in agreement with the "best" values in the literature. The higher results obtained for chymotrypsinogen are discussed.

LITERATURE CITED

(1) Kirk, P. L., "Advances in Protein Chemistry," (edited by Anson, M. L., and Edsall, J. T.) New York **3**, 139 (1947).
(2) Dunn, M. S., and Rockland, L. B., "Advances in Protein Chemistry," (edited by Anson, M. L., and Edsall, J. T.), New York, **3**, 295 (1947).
(3) Chibnall, A. C., Rees, M. W., and Williams, E. F., *Biochem. J.*, **37**, 354 (1943).
(4) Jonnard, R., *Ind. Eng. Chem.*, *Anal. Ed.*, **17**, 246 (1945).
(5) Miller, L., and Houghton, J. A., *J. Biol. Chem.*, **159**, 373 (1945).
(6) Ballentine, R., and Gregg, J. R., *Anal. Chem.*, **19**, 281 (1947).
(7) White, L. M., and Secor, G. E., *Ind. Eng. Chem.*, *Anal. Ed.*, **18**, 457 (1946).
(8) Association of Official Agricultural Chemists, "Official and Tentative Methods of Analysis," 6th ed., sec. **41.5**, p. 763, Washington, D. C. (1945).
(9) Van Slyke, D. D., Hiller, A., and Dillon, R. T., *J. Biol. Chem.*, **146**, 137 (1942).
(10) Vickery, H. B., and Winternitz, J. K., *Ibid.*, **156**, 211 (1944).
(11) Association of Official Agricultural Chemists, "Official and Tentative Methods of Analysis," 6th ed., sec. **2.26**, p. 27, Washington, D. C., (1945).
(12) Van Slyke, L. L., and Baker, J. C., *J. Biol. Chem.*, **35**, 127 (1918).
(13) Kunitz, M., and Northrop, J. H., *J. Gen. Physiol.*, **18**, 433 (1935).
(14) Palmer, A. H., *J. Biol. Chem.*, **104**, 359 (1934).
(15) Alderton, G., and Fevold, H. L., *Ibid.*, **164**, 1 (1946).
(16) Kekwick, R. A., and Cannan, R. K., *Biochem. J.*, **30**, 227 (1936).
(17) Anson, M. L., *J. Gen. Physiol.*, **22**, 79 (1938).
(18) Brand, E., *Ann. N. Y. Acad. Sci.*, **47**, 187 (1946).
(19) Brand, E., and Kassell, B., *J. Gen. Physiol.*, **25**, 167 (1941).
(20) Brand, E., and Kassell, B., *J. Biol. Chem.*, **145**, 365 (1942).
(21) Ogg, C. L., Brand, R. W., and Willits, C. O., *This Journal*, p. 663.
(22) Shirley, R. L., and Becker, W. W., *Ind. Eng. Chem.*, *Anal. Ed.*, **17**, 437 (1945).

MICRO AND SEMIMICRO DETERMINATION OF NITROGEN IN HETEROCYCLIC NITROGEN RING COMPOUNDS BY A KJELDAHL METHOD*

By C. L. Ogg, Ruth W. Brand, and C. O. Willits
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Modifications in the Kjeldahl procedure to increase the range of its application have been numerous, and as Shirley and Becker (8) pointed out, conflicting claims have been made as to the correct procedure for the analysis of compounds containing refractory ring-type nitrogen. Clark (5) claimed that the semimicro adaptation of the Gunning-Arnold-Dyer modification was applicable to practically all animal and vegetable materials, purines, pyrimidines, and pyridine and quinoline derivatives. The Clark modification, which is the tentative micro Kjeldahl method of the Association of Official Agricultural Chemists (2), uses mercuric oxide as catalyst with 1.5 ml concentrated sulfuric acid and 0.5 g potassium sulfate and a total digestion time of 80–85 minutes. However, Clark stated that some alkaloids and related compounds require a longer digestion time, and that atropine and quinine-type compounds require 2 hours' digestion. Acree (1), in doing collaborative work with this method, was able to obtain only one-fourth of the nitrogen in nicotinic acid with a 30-minute digestion and found that a 2-hour digestion was required for strychnine and quinine hydrobromides and atropine sulfate.

Belcher and Godbert (3) reported satisfactory nitrogen values for nicotinic acid, quinolinic acid, atropine sulfate, picolinic acid, etc., by a semimicro method employing mercuric sulfate and metallic selenium as catalysts and a total digestion time of 45 minutes. . . . However, they state that loss of nitrogen occurs when the digestion time exceeds 75 minutes. This loss probably is caused by the high concentration of selenium used in the digestion mixture since the concentration exceeds the limit found by Bradstreet (4). To avoid this loss an empirical digestion time which may vary with different compounds must be used. Miller and Houghton (6), using a micro method with mercuric oxide as catalyst, found that *l*-tryptophane (sample in solution) required a 6-hour digestion, and consequently they adopted this digestion time as standard for proteins and amino acids.

White and Secor (9) reported 98.5 to 100 per cent recovery of the nitrogen from *l*-tryptophane with Clark's method. Although their nitrogen values for *l*-tryptophane with an 85-minute digestion were close to theory, better values were obtained when the time was increased to 115 minutes.

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† One of the Laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture.

Previous to the studies reported in this paper, all compounds containing ring nitrogen were analyzed in this laboratory by the Dumas method. Since so much time is required to condition the Dumas apparatus and establish the blank when it is not in continuous use, and since a number of samples can be run simultaneously by the Kjeldahl method with a considerable saving in time, a reliable micro Kjeldahl procedure for the analysis of such materials is highly desirable. Consequently, a study of the Kjeldahl method was made in which various catalysts and combinations of catalysts were used and the time of digestion ranged from 1 to 6 hours. The semimicro procedure adopted is substantially the same as the macro procedure of Shirley and Becker, and the results obtained in the digestion-time studies on nicotinic acid on the semi-micro and macro scale are relatively parallel. . . . Since the semimicro procedure and apparatus have been successfully applied to micro analyses, both methods are described.

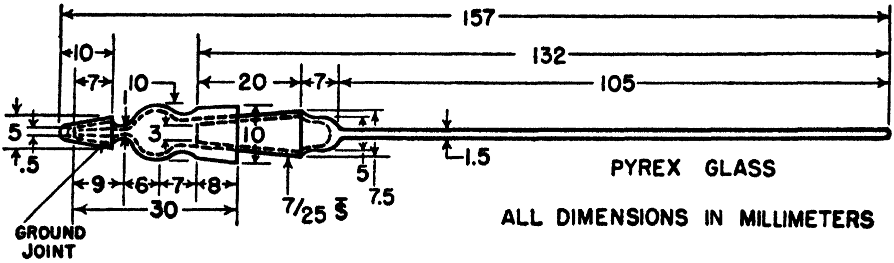

FIG. 1.—Semimicro Charging Tube.

APPARATUS

(1) *Digestion stand.*—6-unit, gas-heated, with glass manifold to allow for the removal of acid fumes by suction from a water aspirator.

(2) *Kjeldahl flasks.*—30 ml Pyrex glass.

(3) *Distillation apparatus.*—Micro Kjeldahl apparatus, Scientific Glass Apparatus Co. Number M-3704.*

(4) *Steam generator.*—A two-liter, three-necked flask with a 24/40 outer joint on the center neck. The flask contains a heating element consisting of a coiled wire of 25-ohms resistance. The ends of the resistance wire are brazed to 14-gage copper leads which pass out through rubber stoppers in the two outer necks and are soldered to the wires of an insulated extension cord. The rate of steam evolution caused by passing a current through the resistance wire is controlled by a variable resistor of 5 ampere rating. With no heat loss between heating element and the water, the heating can be instantaneously controlled.

(5) *Burettes.*—10-ml graduated in 0.05 ml (semimicro) or 5 ml graduated in 0.01 ml (micro).

REAGENTS

(1) *Catalyst.*—Thoroly mix 150 g powdered potassium sulfate, 10 g of mercuric oxide, and 3 g of powdered metallic selenium.

(2) *Sodium hydroxide-sodium thiosulfate soln.*—Mix 100 ml of 50% sodium hydroxide and 25 ml of 8% sodium thiosulfate soln.

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* The mention of commercial products does not imply that they are endorsed or recommended by the Department of Agriculture over others of a similar nature not mentioned.

(3) *Indicator.*—Mix 100 ml of 0.2% methyl red dissolved in 95% ethanol with 50 ml of 0.2% methylene blue also dissolved in 95% ethanol.

(4) *Boric acid.*—Dissolve 4 g boric acid in 100 ml distilled water.

(5) *Standard acids.*—0.02 *N* hydrochloric acid (semimicro) of 0.01 *N* (micro).

PROCEDURE

Weigh a 15–30 mg sample (4–8 mg for the micro procedure) in a long-handled charging tube and transfer to a 30 ml Pyrex Kjeldahl flask. Add 0.65 –0.70 g of the catalyst 3–4 boiling chips, and 2 ml of concd sulfuric acid, rotating the flask as the acid is added so that any sample adhering to the lower portion of the neck will be rinsed into the bulb. Place the flask on the digesting apparatus, heat slowly until carbonization occurs or until all danger of frothing is past, and then adjust the heat so that the sulfuric acid distills ca two-thirds the way up the neck of the flask. Continue the digestion for 4 hours for materials containing ring nitrogen, or 1 hour for more easily digested material such as amines or amides. After the digestion is complete, cool the flask, add 3 ml of water, and again cool the flask to room temp. Condition the distillation apparatus by steaming it out for 2–3 min., and then rinse it with distilled water. Place 10 ml of a 4% boric acid absorbing soln and 3 drops of indicator in a freshly rinsed 125 ml Erlenmeyer flask and place this flask under the tube of the condenser so that the delivery tip is immersed in the soln. Transfer the digestion mixture to the distillation unit with both stopcocks open to prevent any sample from being drawn into the outer chamber. To insure quantitative transfer, rinse the flask with four 2 ml portions of distilled water, or until the rinsings remain yellow on addition of a drop of methyl orange indicator. Add 6 ml of the sodium hydroxide-sodium thiosulfate soln (1 ml in excess of that needed to neutralize the acid), close both stopcocks, and heat the water in the steam generator slowly until the displaced air has mixed the acid and alkali. If the generator is heated too rapidly, the violent reaction which will result from the sudden mixing of the acid and alkali may cause the sample and absorbing soln to be sucked back thru the apparatus. After the acid and alkali are thoroly mixed, turn the current on full to generate the steam at a rapid rate until it reaches the condenser, then reduce the rate so that the steam does not pass the upper half of the condenser. (This should allow 3–4 ml of distillate to be collected per min.) After it has distilled for 3 min., lower the receiving flask so that the delivery tip is above the soln and continue the distillation for 1 min. to rinse the inside of the condenser tube. Rinse the outside of the delivery tip with distd water, dilute the soln to 50 ± 5 ml, and titrate with the appropriate standard hydrochloric acid until the soln changes from green to gray or gray-purple. Run a blank determination to correct for nitrogen in the reagents. Use this blank value to correct the volume of acid used in titrating the sample and calculate the percentage of nitrogen.

(Ten milliliters of the 4% boric acid soln, after dilution to 50 ml, is slightly acid to the indicator, but in practice the ammonia from the reagents "blank" has always been sufficient to neutralize the boric acid.)

Hygroscopic solids are dried and weighed in long-handled charging tubes (7) (Figure 1). The tube and cap are made from a 7/25 inner and outer joint with 10 mm of the outer joint (large cap) cut off to reduce the weight. Sealed to the top of the large cap is a smaller inner joint with a capillary opening, which in turn is covered by a ground-glass cap. The sample is placed in the charging tube and dried under vacuum, with only the large cap attached. When the sample is dry, the vacuum is released by admitting dry air to the drying chamber. The charging tube is then

TABLE 1.—*Effect of catalyst and digestion time in the Kjeldahl analyses of nicotinic acid*
Nicotinic Acid, Theory = 11.38% *N*

CATALYST	PER CENT NITROGEN FOUND AFTER DIGESTION TIME OF:											
	1 HOUR			2 HOURS			3 HOURS			4 HOURS		
	Max.	*Min*	*Ave.*	*Max.*	*Min.*	*Ave.*	*Max.*	*Min.*	*Ave.*	*Max.*	*Min.*	*Ave.*
Hg (1 drop)				8.69	7.86	8.33						
HgO (40 mg)				9.30	8.40	8.85	9.91	8.66	9.54	11.26	10.78	11.00
HgO+Se[1]	7.40	5.68	6.54	10.16	9.36	9.74	11.39	10.33	11.08	11.39	11.28	11.36
$CuSO_4$[2]+Se				8.42	7.28	7.95						
HgO+$SeOCl_2$ (1 drop)	4.98	4.88	4.93	8.82	7.22	8.26	11.02	8.10	9.66	11.35	11.28	11.31
HgO+$SeOCl_2$ (2 drops)				11.12	8.01	10.04	10.76	10.39	10.58			
$CuSO_4$+$SeOCl_2$ (1 drop)				7.54	6.11	6.58						
HgO+Se+$CuSO_4$	5.55	4.55	5.05	10.34	7.78	8.99				11.28	11.07	11.16
HgO+$CuSeO_3$ (5 mg)				8.42	7.28	7.95						
Clark's Method (5)	5.31	3.58	4.45									

[1] Selenium-coated granule.
[2] 0.15 g. $CuSO_4 \cdot 5H_2O$.

removed, and the capillary is immediately closed with the small cap and allowed to come to room temperature before weighing.

Moderately volatile or hydroscopic liquid samples are weighed in capsules made by sealing the tube of a 5/12 inner joint just below the joint. For weighing and introducing into the digestion flask the capsule is inserted loosely in a holder consisting of a 5/12 outer joint whose tube serves as a handle. After the capsule and holder are tared, the sample is introduced in the capsule with either a hypodermic syringe or a medicine dropper having a capillary tip, and the capsule is placed in the holder. The capsule with the sample is weighed and dropped into a digestion flask containing the acid and catalyst by gently tapping the holder against the neck of the flask. After digestion, the mixture is transferred to the distilling apparatus and the flask is rinsed as usual. The capsule, which is caught in the funnel, is removed with tweezers, held in an inverted position over the funnel, and rinsed with 2 ml of water from a hypodermic syringe. The needle is bent in a V-shape so that the rinse solution which drains down is transferred to the funnel. Very viscous liquids, gums and resins are introduced into the flask in porcelain boats. Hygroscopic samples are dried and weighed in weighing pigs.

RESULTS

Nicotinic acid was chosen for use in studies of catalyst and digestion time, since it contains a ring-type nitrogen which has proved difficult to obtain by the Kjeldahl method, is easily obtained in pure form, and is a non-hygroscopic solid. Several catalysts and combinations of catalysts were used, and the digestion time was varied from 1 to 4 hours. . . . The amount of catalyst in each combination was the same unless otherwise noted. Table 1 shows the results obtained with the various digesting conditions.

A 2-hour digestion period was used in comparing the relative effectiveness of the various catalysts and combinations, since it was not sufficiently long to give complete recovery with any of the catalysts. The results obtained under these conditions indicate the most effective catalyst to be a combination of mercuric oxide and selenium. Mercuric oxide and selenium oxychloride (2 drops) gave a higher nitrogen value in 2 hours than did the mercuric oxide and selenium, but as Bradstreet (4) has shown, the ratio of selenium to sulfuric acid was so high that loss of nitrogen was almost certain, and as expected, the values obtained after the 3-hour digestion were lower than those obtained with mercury and selenium. Increasing the amount of selenium increased the rate of conversion of nitrogen to ammonia, but apparently nitrogen was lost when the selenium content was raised much above 10 mg per ml of sulfuric acid. The results obtained for the 3-hour digestion with mercuric oxide and selenium show that in some cases theoretical values were obtained but the recoveries were not

TABLE 2.—*Kjeldahl nitrogen analyses of several nitrogenous compounds*

COMPOUND	CATALYST	DIGESTION TIME	NITROGEN			
			FOUND		AVE.	THEORETICAL
		hours	*per cent*	*per cent*	*per cent*	*per cent*
N (α-pyridyl) nicotinamide	HgO+Se	3	20.80			
		3	20.85	20.67	20.77	21.10
		4	21.02	21.03		
		4	21.02	21.07	21.04	21.10
Nicotine	HgO+Se	1	13.74	14.79	14.27	17.27
		2	16.93	16.46	16.70	17.27
		3	17.22	17.23		
		3	17.31	17.19	17.24	17.27
		6	17.20	17.22	17.21	17.27
8-Hydroquinoline	HgO+Se	4	9.64	9.69	9.67	9.65
Tryptophane	HgO+$SeOCl_2$	4	13.74	13.72		
		4	13.75	13.77	13.75	13.72
Tryptophane	HgO+Se	1	13.17	13.05	13.11	13.72
		2	13.75	13.70	13.73	13.72
		4	13.65	13.66		
		4	13.64	13.69	13.67	13.72
Acetonicotyrine	HgO+$SeOCl_2$	4	11.34	12.88	12.11	13.73
		6	13.66	13.51	13.59	13.73
Nicotine picrate[1]	HgO+Se	4	18.11	18.05		
		4	17.89	18.16	18.05	18.06
Nicotine picrate[2]	HgO+Se	4	18.01	17.98	18.00	18.06
S-benzyl thiuronium chloride	HgO+Se	1	13.78	13.83		
		1	13.79	13.79	13.80	13.82
Acetanilide	HgO+Se	1	10.35	10.31	10.33	10.36

[1] Sample treated with salicylic acid and sodium thiosulfate. 3 ml. H_2SO_4 used.

[2] Sample digested for 2 hours with carbon prior to regular 4-hour digestion. 3 ml. H_2SO_4 used.

consistent, whereas with 4 hours' digestion concordant results were obtained which were close to the theoretical values. With the catalyst mixture recommended complete recovery of the nitrogen was obtained in 4 hours and no loss of nitrogen was detected with a 6-hour digestion. Consequently, a 4-hour digestion period was tentatively adopted as standard for all materials containing ring-type nitrogen. This digestion time is 1 hour longer than was indicated by Shirley and Becker's work (8), in which macro procedures were used. The difference may be due to the type of heater used during the digestion, that is, electrical, versus gas heaters. Nitrogen was determined in several materials in addition to nicotinic acid by the method described to test its applicability to several types of compounds having ring nitrogen. The results are reported in Table 2.

All the compounds studied yielded their nitrogen after a 4-hour digestion with mercuric oxide and selenium except acetonicotyrine, which required a 6-hour digestion for the values to approach theory.

Nicotine, whose structure differs only slightly from that of nicotyrine, needed only a 3-hour digestion, thus indicating the difficulty that may be experienced in attempting to predict the digestion time required from the structure of the compound to be analyzed. Doubling the digestion time required for nicotine did not significantly lower the nitrogen values, proving that if loss of nitrogen does result from the use of selenium catalyst it is negligible. As would be expected, a 1-hour digestion was sufficient for acetanilide and S-benzyl thiuronium chloride, since they do not contain ring nitrogen. Tryptophane yielded its nitrogen after a 2-hour digestion. This is in agreement with the observations of White and Secor (9).

The results obtained show that in most cases a 4-hour digestion with mercuric oxide and selenium as catalysts is sufficiently long to obtain the nitrogen from heterocyclic nitrogen ring compounds. Apparently a few compounds require at least a 6-hour digestion. It is easy to detect these materials, however, since the incomplete 4-hour digestion usually is accompanied by discordant results.

SUMMARY

A semimicro Kjeldahl method is presented for the determination of nitrogen in compounds containing heterocyclic ring nitrogen. This method has proved successful in the analysis of micro-sized samples. With a mixture of mercury and selenium, the best catalyst found, a minimum digestion time of 4 hours is required. For compounds not containing ring nitrogen, the digestion time can be shortened to 1 hour.

LITERATURE CITED

(1) Acree, Fred J., *This Journal*, **24**, 648 (1941).
(2) *A.O.A.C.*, *Official and Tentative Methods of Analysis*, 6th Ed., p. 763 (1945).
(3) Belcher, R., and Godbert, A. L., *J. Soc. Chem. Ind.*, **60**, 196 (1941).
(4) Bradstreet, R. B., *Ind. Eng. Chem., Anal. Ed.*, **12**, 657 (1940).
(5) Clark, E. P., *This Journal*, **24**, 641 (1941).
(6) Miller, Lila, and Houghton, Jean Anne, *J. Biol. Chem.*, **159**, 373 (1945).
(7) Porter, W. L., Brice, B. A., Copley, M. J., and Couch, J. F., U.S.D.A. Bur. Agr. and Ind. Chem. AIC-159, 1947. (Processed.)
(8) Shirley, Ray L., and Becker, W. W., *Ind. Eng. Chem., Anal. Ed.*, **17**, 437 (1945).
(9) White, Lawrence M., and Secor, Geraldine E., *Ibid.*, **18**, 457 (1946).

THE ANALYSIS OF LANOLIN-HYDROCARBON MIXTURES*

By S. H. NEWBURGER (Cosmetic Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.)

The analysis of lanolin-hydrocarbon mixtures is of interest because hydrocarbons are often an adulterant in lanolin and because hydrocarbons are a constituent of lanolin absorption bases. In a recent article in *The Analyst*, Williams[1] states that he separates hydrocarbons from wool grease by chromatographing on an aluminum oxide column with petroleum benzin as the solvent. However, no data or experimental details were given. This investigation was undertaken to determine the conditions under which this separation could be effected.

METHOD

PREPARATION OF Al_2O_3 COLUMN

Prepare an activated Al_2O_3 column 9″×¾″ in the following manner: Tamp a ¾″ cotton plug into the bottom of the chromatograph tube, fill the tube with petroleum benzin, and pour enough Al_2O_3 ("Alorco" grade F-20 mesh 80–200) thru the petroleum benzin to form a column about 1½″ high. Pack the Al_2O_3 by applying an air pressure of 5 or 6 pounds to the chromatograph tube. Then add successive increments of Al_2O_3 in this manner until the column totals a length of about 9″. Finally, place another cotton plug on top of the column.

CHROMATOGRAPHIC PROCEDURE

Dissolve sample in 50 ml warm petroleum benzin (B.P. 30-75°C.), cool the soln to room temp., transfer to chromatograph tube with the aid of 25 ml of petroleum benzin, and allow the soln to flow by gravity thru the column at a rate of 3.3 to 6 ml per min. Follow this soln with 175 ml of petroleum benzin, combine the two filtrates and label them as filtrate No. 1. Put another 50 ml of petroleum benzin thru the column and label this filtrate as filtrate No. 2. Evaporate the filtrates on the steam bath, dry the residues in an oven at 100°C. for 10 min., cool in a vacuum desiccator, and weigh. Repeat drying in oven until weight is constant to 1 mg.

The first experiments tested the adsorbability of lanolin on the column. Samples of U.S.P. anhydrous lanolin varying from 2.5 to 6.6 grams were chromatographed. All samples larger than 3 grams gave an appreciable residue in both filtrates. Samples less than 3 grams gave a small residue in the first filtrate and a negligible residue in the second filtrate. The data are tabulated in Table 1.

As lanolin is a complex natural product of variable composition, 3 gram samples of several commercial products including one of hydrous lanolin were chromatographed. The results are presented in Table 2.

The retention of all the lanolin samples on the aluminum oxide was greater than 99 per cent.

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* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 20–22, 1947.

[1] Williams, K. A., *Analyst*, 71, 259 (1946).

A 5.230 gram sample of U.S.P. mineral oil was then dissolved in petroleum benzin and chromatographed by the procedure described for lanolin. The rate of flow of the solution was 4 ml per minute. About 99.9 per cent of the mineral oil came through in the first filtrate.

Mixtures of mineral oil with anhydrous lanolin (Product "a") and with hydrous lanolin, the total weight of each mixed sample not exceeding 3 grams, were prepared and chromatographed. The data are given in Table 3.

TABLE 1.—*The adsorption of lanolin on aluminum oxide*

SAMPLE U.S.P. ANHYDROUS LANOLIN PRODUCT "A"	RATE OF FLOW PETROLEUM BENZIN	RESIDUE FROM FIRST FILTRATE	RESIDUE FROM SECOND FILTRATE
gm	*ml per minute*	*gm*	*gm*
6.625	3.3	1.894	0.068
5.027	4.0	0.674	0.088
4.014	4.6	0.098	0.066
3.110	4.0	0.014 (0.5%)	0.000
3.024	5.5	0.014 (0.5%)	0.000
2.505	4.5	0.013 (0.5%)	0.000

TABLE 2.—*Variations in adsorbability of lanolin samples*

SAMPLE		RATE OF FLOW PETROLEUM BENZIN	RESIDUE FROM FIRST FILTRATE	RESIDUE FROM SECOND FILTRATE
	gms	*ml per minute*	*per cent*	*per cent*
Product "a" . . . . (U.S.P. Anhydrous Lanolin)	3.024	5.5	0.5	0.0
Product "b" . . . . (U.S.P. Anhydrous Lanolin)	2.998	3.3	0.3	0.0
Product "c" . . . . (U.S.P. Anhydrous Lanolin)	2.999	4.0	0.8	0.0
Product "d" . . . . (U.S.P. Hydrous Lanolin)	3.018	4.5	0.3	0.0

The values for the unadsorbed material are greater than the percentages of mineral oil; this is to be expected as the lanolins have blanks of 0.5 and 0.3 per cent. If the lanolin blanks are subtracted, the percentages of mineral oil are in good agreement with the unadsorbed matter.

A sample of U.S.P. solid petrolatum was then chromatographed. Only 92.6 per cent came through in the first filtrate and an additional 1 per cent in the second filtrate (Table 4). Approximately the same behavior was observed with a sample of vaseline. Neither of these samples had any appreciable volatile matter at 100°C. As shown in the table, a third filtrate of another 50 ml petroleum benzin was also collected.

Mineral oil is a distilled product and is often purified with fuming sulfuric acid. The end product is almost wholly saturated hydrocarbons.

TABLE 3.—*The chromatography of mixtures of mineral oil and lanolin*

SAMPLE		RATE OF FLOW PETROLEUM BENZIN	RESIDUE FROM FIRST FILTRATE	RESIDUE FROM SECOND FILTRATE	MINERAL OIL ADDED	UNADSORBED MATERIAL
	gm	*ml per minute*	*gm*	*gm*	*per cent*	*per cent*
U.S.P. Anhydrous lanolin....	2.733	3.5	0.110	0.000	3.4	3.9
U.S.P. Heavy mineral oil....	0.096					
Total.............	2.829					
U.S.P. Anhydrous lanolin....	2.637	4.0	0.311	0.001	10.2	10.6
U.S.P. Heavy mineral oil....	0.300					
Total.................	2.937					
U.S.P. Anhydrous lanolin....	2.250	3.5	0.732	0.000	24.2	24.7
U.S.P. Heavy mineral oil....	0.719					
Total.................	2.969					
U.S.P. Anhydrous lanolin....	1.453	4.5	1.532	0.002	51.3	51.4
U.S.P. Heavy mineral oil....	1.528					
Total.................	2.981					
U.S.P. Hydrous lanolin......	2.506	4.5	0.296	0.000	10.4	10.6
U.S.P. Heavy mineral oil....	0.290					
Total.................	2.796					

TABLE 4.—*The chromatography of solid petrolatum*

SAMPLE		RATE OF FLOW PETROLEUM BENZIN	RESIDUE FROM FIRST FILTRATE	RESIDUE FROM SECOND FILTRATE	RESIDUE FROM THIRD FILTRATE
	gm	*ml per minute*	*per cent*	*per cent*	*per cent*
U.S.P. Solid petrolatum.	2.983	3.5	92.6	1.0	0.7
Vaseline..............	2.316	4.4	91.2	1.2	0.7

Petrolatum, on the other hand, is a residue and contains unsaturated hydrocarbons as well as their oxidation products. It is probably this material which is held on the column.

TABLE 5.—*The chromatography of mixtures of lanolin and solid petrolatum*

SAMPLE		RATE OF FLOW PETROLEUM BENZIN	RESIDUE FROM FIRST FILTRATE	RESIDUE FROM SECOND FILTRATE	PETROLATUM ADDED	UNADSORBED MATERIAL
	gm	*ml per min.*	*per cent*	*per cent*	*per cent*	*per cent*
U.S.P. Anhydrous lanolin.......	2.905	6.0	2.9	0.0	2.6	2.9
U.S.P. Solid petrolatum........	0.077					
Total....................	2.982					
U.S.P. Anhydrous lanolin......	2.660	4.5	9.8	0.1	10.1	9.9
U.S.P. Solid petrolatum.......	0.298					
Total....................	2.958					
U.S.P. Anhydrous lanolin..... ..	1.894	5.0	32.1	0.4	34.1	32.5
U.S.P. Solid petrolatum........	0.982					
Total	2.876					
U.S.P. Anhydrous lanolin.......	1.414	4.0	48.8	0.5	52.2	49.3
U.S.P. Solid petrolatum........	1.545					
Total....................	2.959					
U.S.P. Anhydrous lanolin.......	0.426	4.5	79.4	0.9	85.6	80.3
U.S.P. Solid petrolatum........	2.524					
Total......	2.950					

Mixtures of anhydrous lanolin (Product "a") and solid petrolatum were prepared and chromatographed. The data are presented in Table 5.

The values for the unadsorbed material are the sum of the residues from both filtrates. However, the residue from the second filtrate was never greater than 0.9 per cent and for mixtures containing less than 10 per cent petrolatum it was not significant. The unadsorbed material calculated as petrolatum recoveries does not give values as good as those obtained for mineral oil; however, if the 0.5 per cent blank is subtracted from the unadsorbed material recovered, the resulting values all fall within 1.3 per cent of the 93.6 per cent unadsorbed material expected from the petrolatum.

SUMMARY

Under the described experimental conditions hydrous and anhydrous lanolin are adsorbed on the aluminum oxide to an extent greater than 99 per cent. Hydrocarbons are not adsorbed at all. Advantage is taken of these phenomena to separate mineral oil and solid petrolatum from lanolin.

SPECTROPHOTOMETRIC TESTING OF D&C VIOLET NO. 2

(ALIZUROL PURPLE SS)

By MEYER DOLINSKY (Cosmetic Division, Food and Drug Administration, Federal Security Agency, Washngton, D. C.)

The pure dye content of samples of D&C Violet No. 2 is usually determined either by titration with titanium trichloride, or by calculation from the nitrogen content of the sample. Neither of these methods is satisfactory. The end point of the titration is difficult to detect, since the reduction products are also colored. Calculation of the pure color from the nitrogen content of the sample will give high results when nitrogen containing impurities are present.

It was therefore decided to investigate a spectrophotometric method for the determination, following the method of Clark and Newburger on the Spectrophotometric Analysis of Coal-Tar Colors.[1]

EXPERIMENTAL

Preparation of Standard Sample.—Quinizarin (1, 4-dihydroxyanthraquinone), m.p. 194°C. (literature 194°C.), was reduced to "leuco-quinizarin" by means of stannous chloride plus hydrochloric acid.[2] The product, after recrystallization from benzene, melted at 155°C. (literature 150°–155°C.). This was then condensed with p-toluidine,[3] m.p. 43°C. (literature 42.8°–45°C.), The dye, after repeated crystallization from glacial acetic acid, melted at 190°C. (literature 183°C.). A further recrystallization of a portion of the product from a chloroform-alcohol mixture showed no change in melting point or spectrophotometric characteristics.

Nitrogen determination by a semimicro Kjeldahl method gave a pure dye content for the product of 100.4 per cent; titration with titanium trichloride, using di-sodium citrate as a buffer, gave a pure dye content of 99.5 per cent.

Preparation of Solutions.—A 31.02 mg sample of dye, weighed on a semimicro balance, was dissolved in sufficient U.S.P. chloroform to make the volume exactly 100 ml. Aliquot portions of this solution were then diluted with chloroform to give the concentrations shown in Table 1.

The chloroform solution of D&C Violet No. 2 (Chart 1) shows a characteristic flattened absorption curve with a peak at 587 ± 2 mμ. The ratio of E_{650} mμ/E_{600} mμ was found to be 1.00 ± 0.02. Deviations from Beer's law at 587 mμ are less than 1 per cent.

All optical measurements were made with a General Electric recording spectrophotometer using an 8 mμ slit. Melting points were taken on a Fisher M. P. Block.

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[1] G. R. Clark and S. H. Newburger, "Spectrophotometric Analysis of Coal-Tar Colors I. Ext. D&C Yellow No. 5." *This Journal*, 27, 576 (1944).

[2] Meyer and Sander, *Ann.*, 420, 122 (1920).

[3] Friedlander and Schick, *Chem. Zentr.*, 1904, II, 339.

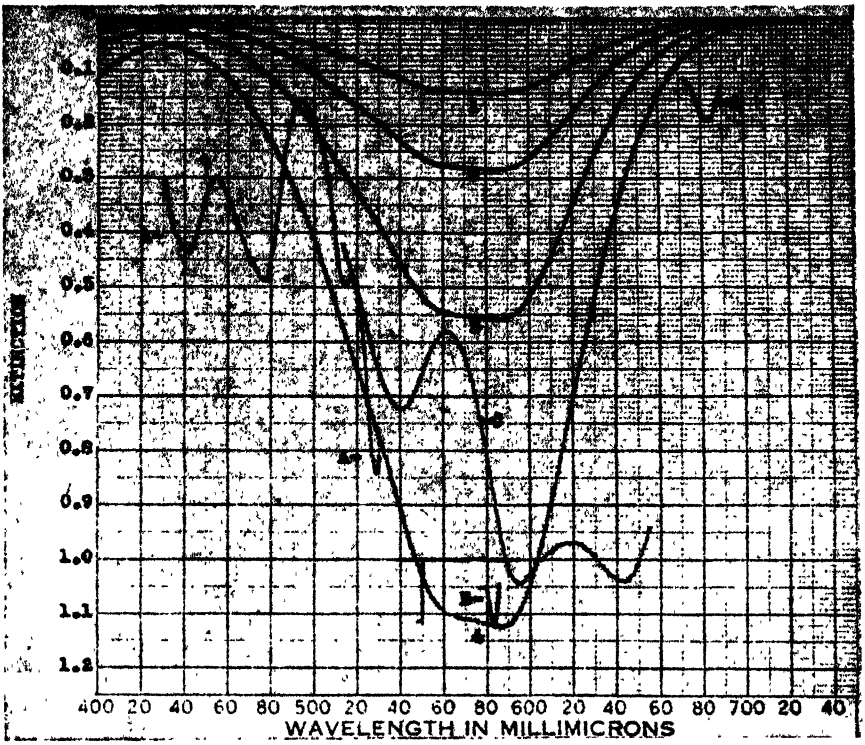

CHART 1.—Pure D&C Violet No. 2 in chloroform.
Curve 1— 3.88 mg./liter.
Curve 2— 7.76 mg./liter.
Curve 3—15.51 mg./liter.
Curve 4—31.02 mg./liter.
Cells—1 cm.
A—Corning Didymium Glass 512, 6.0 mm.
(Absorption peaks at 400.4, 441.4, 477.1, 529.0, and 684.8 mμ.)
B—Corning Didymium Glass 592, 4.02 mm.
(Absorption peak at 583.7 mμ.)
C—Signal Lunar White Glass H-6946236.

TABLE 1.—*Extinction values of chloroform solutions of purified D&C Violet No. 2*

CURVE NO. (CHART 1)	CONCENTRATION	E_{587} m μ	$\frac{E_{587}\ m\mu}{\text{CONCENTRATION}}$
	mg/liter		
1	3.88	0.140	0.0361
2	7.76	0.282	0.0363
3	15.51	0.558	0.0360
4	31.02	1.123	0.0362
			Av. 0.0362

APPLICATION TO COMMERCIAL SAMPLES

A commercial sample of D&C Violet No. 2 was analyzed spectrophotometrically, following the procedure described for the standard sample. The curve is shown on Chart 2 and the data in Table 2.

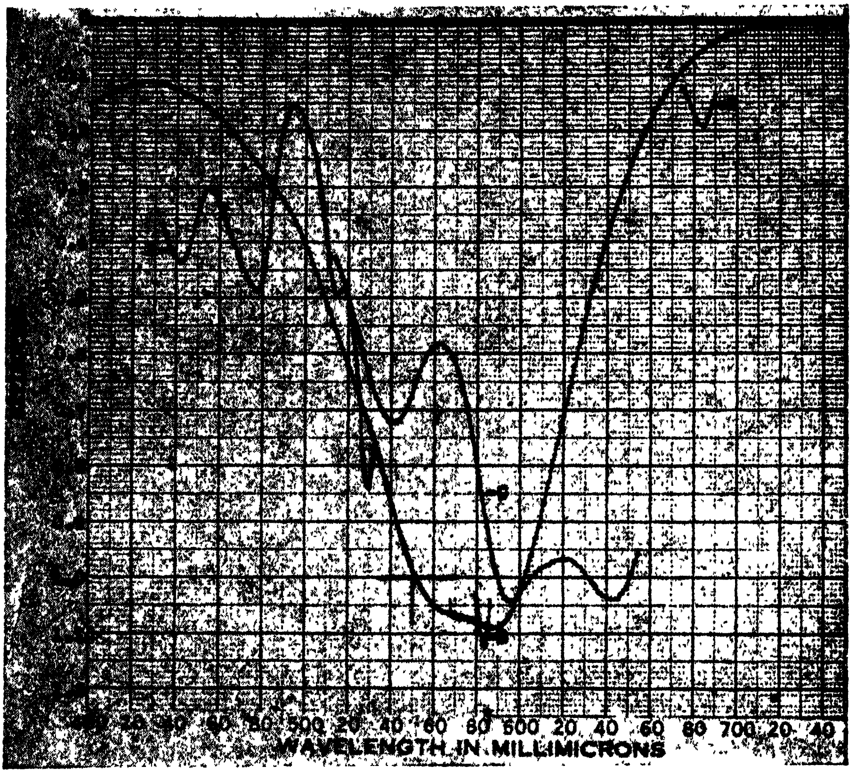

Chart 2.—Commercial Sample of D&C Violet No. 2 in chloroform.
Curve 1—31.90 mg./liter.
Cells—1 cm.
A—Corning Didymium Glass 512, 6.0 mm.
(Absorption peaks at 400.4, 441.4, 477.1, 529.0, and 684.8 mμ.)
B—Corning Didymium Glass 592, 4.02 mm.
(Absorption peak at 583.7 mμ.)
C—Signal Lunar White Glass H-6946236.

Table 2.—*Analysis of commercial sample of D&C Violet No.* 2

CONCENTRATION	E_{587} mμ (IN $CHCl_3$)	DYE SPECTROPHOTOMETRICALLY	DYE BY TITRATION WITH $TiCl_3$	DYE FROM N_2 CONTENT
mg/liter		*per cent*	*per cent*	*per cent*
31.90	1.089	94.3	93.4	98

A portion of this commercial sample after several recrystallizations from glacial acetic acid gave an extinction per milligram of 0.0362, corresponding to a pure dye content of 100 per cent by comparison with the laboratory sample.

SUMMARY

Spectrophotometric data for chloroform solutions of purified D&C Violet No. 2 are presented. Beer's law is shown to be applicable; the extinction per milligram/liter is 0.0362 ± 0.0002 at the absorption peak, 587 ± 2 mμ; the extinction ratio E_{560} mμ/E_{600} mμ is 1.00 ± 0.02.

Application is made of these data to the determination of pure dye in a commercial sample of the color.

DETERMINATION OF LEAD IN LAKES OF COAL-TAR COLORS

By Lee S. Harrow (Food and Drug Administration,
Federal Security Agency, Washington, D. C.)

Studies of several methods for the determination of small amounts of lead in foods and biological materials have been reported by Wichmann *et al.* (1), Clifford (2), and others. The dithizone method for estimating lead in foods has been adopted as tentative in the "Official and Tentative Methods of Analysis," A.O.A.C., Sixth Edition (1945) **29.34.** Ettelstein (3) has reported the application of this method to the determination of lead in straight coal-tar colors. However, the determination of lead in lakes of coal-tar color offers additional problems. In the study to be described, separate methods were developed for aluminum lakes extended upon alumina, and for lakes containing barium and calcium.

ALUMINUM LAKES

Under the conditions usually employed in the extraction of lead as the dithizone complex from aqueous solutions, aluminum hydroxide is precipitated when considerable quantities of aluminum are present. Clifford (2) has shown that lead is occluded with the precipitate, and that the occluded lead is not recovered by the dithizone separation process even when a large amount of citric acid is used.

Table 1 is a tabulation of typical coal-tar color aluminum lakes showing the approximate percentages of aluminum oxide.

In order to eliminate these two possible sources of error, the *Methods of Analysis* (4) offers a means of separating the lead as lead sulfide from the interfering substances. The procedure consists essentially of precipitating the lead as lead sulfide at a *p*H of 3.0–3.4, filtering and dissolving the sulfides with hot concentrated nitric acid, then extracting the lead with di-

TABLE 1.—*Typical aluminum lakes*

COLOR LAKES	ALUMINUM OXIDE
	per cent
FD&C Blue No. 1	87
FD&C Red No. 1	58
FD&C Yellow No. 5	85
FD&C Yellow No. 6	83
D&C Green No. 1	88

thizone solution. This method was modified to make it applicable to aluminum lakes of coal-tar colors.

EXPERIMENTAL

APPARATUS

The apparatus for the operation as shown in Figure 1 consists of a precipitation tube fitted with various inlets to permit introduction of the sample, introduction of hydrogen sulfide, release of excess hydrogen sulfide, and transfer of the precipitated lead sulfide to the filter.

Careful preparation of the filter is essential in order to allow rapid filtration with complete retention of the precipitate. "Celite" or other similar filter aid placed on a fine porosity sintered glass filter gives satisfactory results. For quantities of lead between 20 and 300 micrograms, approximately 0.5 gram of the filter aid should be placed on the filter and washed thoroughly, first with concentrated nitric acid, then with water before use.

Particular attention should be paid to the cleanliness of the apparatus. In this work all glassware was washed successively with scouring powder, tap water, concentrated nitric acid, and distilled water. The precipitation apparatus was washed with concentrated nitric acid and water after each run.

REAGENTS

All reagents should be lead free (5). Any convenient source of H_2S may be used. The gas should be scrubbed first with (1:1) H_2SO_4 (v/v), then with distilled water, before being passed into the solution.

METHOD

Weigh 2 g of the sample into a 500 ml Kjeldahl digestion flask, add 10 ml of conc. H_2SO_4 and 10 ml of conc. HNO_3, and digest on a low flame until SO_3 fumes appear. Add successive 5 ml portions of conc. HNO_3 (waiting until SO_3 fumes appear before adding each succeeding portion) until all organic matter is in solution. Slowly introduce 5–10 ml of a (1:1) mixture of conc. HNO_3 and 60–70% $HClO_4$, and continue the digestion until the white ppt. formed showed the first signs of spattering. Allow the flask to cool and cautiously add 5 ml of H_2O and then a few drops of conc. NH_4OH. Swirl the flask vigorously and cool under running water. Add 20 ml of 50% (w/v) citric acid soln and adjust the *p*H to 3.0–3.4 (bromophenol blue) with conc. NH_4OH. Add 1 ml of $CuSO_4$ soln containing 1 mg Cu per ml and transfer the soln to the pptn tube (B) of the sulfiding apparatus. Bubble H_2S thru the soln at a rate of ca 2 bubbles per sec. for 3–5 min. and filter the resulting suspension thru (C) at a rate of ca 1 drop per sec. When filtration is complete remove the receiver containing the filtrate and attach a suction test tube as shown in Figure 1 (E). Add 3 ml of hot conc. HNO_3 thru the separatory funnel (A) and draw thru the filter, followed with 2 ml of hot water. Detach the filter and pass an additional 3 ml of hot conc. HNO_3 thru the filter, wetting all sides. Again follow with 2 ml of hot water. If the filter is still colored with PbS, wash again with hot conc. HNO_3 and water. Wash the dissolved sulfides into the pptn tube (B), wetting all sides to take up any residual lead sulfide and then into a 50–100 ml glass-stoppered conical flask. Stopper and shake for a few sec., then remove the stopper and boil until the soln clears, to remove the last traces of H_2S and to coagulate any free sulfur present.

Transfer the soln to a 250 ml separatory funnel. Wash the flask with two 5 ml portions of distilled water and add the washings to the main soln. Add 10 ml of 50% (w/v) citric acid soln, 5 ml of 10% sodium cyanide soln, a few drops of hydroxylamine hydrochloride soln to prevent oxidation of the dithizone, adjust the *p*H to 8.5–9.5 (thymol blue) with conc. NH_4OH and proceed with the dithizone extraction and electrolysis as described in the A.O.A.C. Book of Methods (6).

RESULTS

The method was used to determine lead in a composite sample of D&C Blue No. 1, Aluminum Lake, containing approximately 88% of aluminum oxide. Quantities of lead ranging from 20 parts per million to 150 parts per million were added to 2 gram samples. Recoveries obtained are given in Table 2. As shown in Table 2, an average recovery of 99 per cent was obtained.

DISCUSSION

The success of the method depends for the most part on the complete solution of the digestion mixture upon neutralization. The following conditions were noted as being conducive to complete solution:

(1) The neutralization should be carried out slowly and with vigorous agitation to allow action of the ammonium hydroxide on the solid material. As the *p*H of 3.0 is approached, the solution should be allowed to heat up. Fifteen to twenty minutes is usually a sufficient time for the addition of the ammonium hydroxide if the solution has been adequately heated and swirled.

(2) If at the completion of the neutralization the solution contains solid material or is translucent, allow the solution to stand about 10 minutes. This usually brings about complete solution.

The color changes of bromophenol blue range from yellow at *p*H 3.0 through olive to purple at 3.4 or above. The *p*H range for the quantitative precipitation of lead sulfide without co-precipitation of ferrous sulfide is from 2.5–3.4 (orange-yellow with thymol blue to incipient purple or olive-green with bromophenol blue). Adjustments should be made only from the acid side to avoid any permanent precipitation of the hydroxides. (Addition of citric acid, cooling of the iron solution, and agitation should prevent the precipitation of aluminum, iron, or alkaline earth hydroxides.) When enough iron is present to mask the color, final adjustments of the pH should be made on a spot plate.

Copper acts as a good collector of lead and also as a filter aid (2). (It has been found that for a quantity of lead ranging from 10 to 300 micrograms, 1–5 mg. should be added.)

TABLE 2.—*Recovery of lead*

BLANK* MICROGRAMS Pb	Pb ADDED	EQUIVALENT TO PER CENT	Pb RECOVERED	NET RECOVERY	PER CENT	AVERAGE PER CENT
	micrograms		*micrograms*	*micrograms*		
20	40	0.0020	57	37	93	97
			59	39	98	
			58	38	95	
			59	39	98	
			60	40	100	
27	100	0.0050	124	97	97	99
			129	102	102	
			127	100	100	
			127	100	100	
			125	98	98	
29	160	0.0080	190	159	99	99
			193	162	101	
			190	159	99	
			188	160	100	
			186	158	99	
28	200	0.0100	227	200	100	97
			219	192	96	
			228	201	100	
			230	202	101	
			227	199	100	
28	300	0.0150	325	297	99	100
			328	300	100	
			329	301	100	
			327	299	100	
			335	308	103	
			328	300	100	

* The blank included the electrode blank, reagent blank, and dye without added lead.

CONCLUSION

The method described above appears to give accurate and reproducible results.

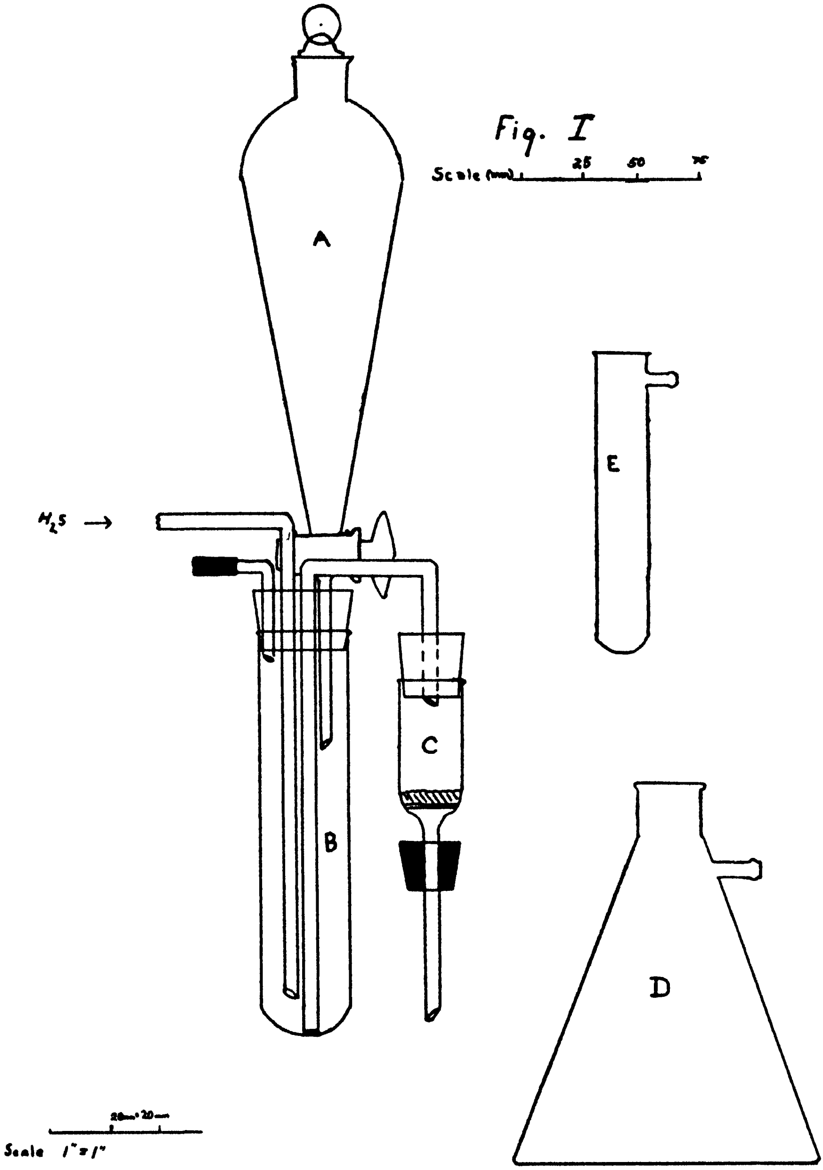

Fig. I

BARIUM, CALCIUM, AND STRONTIUM LAKES

The usual wet digestion methods cannot be used for the decomposition of alkaline earth lakes because of the insolubility of the digestion residue (calcium, barium, or strontium sulfates) in alkaline solution. Determination of lead in cosmetics by a dithizone extraction method following fusion with an excess of sodium and potassium carbonates has been described by

Kress (7). The method described herein represents a modification of this procedure applicable to the analysis of color lakes.

EXPERIMENTAL

REAGENTS

Sodium carbonate.—Lead free, analytical grade.
Potassium carbonate.—Lead free, analytical grade.
Sodium nitrate.—Lead free, analytical grade.
Sodium carbonate soln.—5% (w/v).
Hydrochloric acid.—(2:5) (v/v).

METHOD

Place 2 g of the lake, 4 g of Na_2CO_3, 6 g of K_2CO_3, and 0.5 g of $NaNO_3$ in a platinum crucible of suitable size. Mix thoroly. Heat carefully until the color is carbonized, then heat to about 850°C. and hold at that temp. for 15 minutes. If a controlled muffle furnace is available, it is only necessary to place the fusion mixture in the cold furnace and raise the temp. gradually to 850°C. over a two-hour period. Usually 15–30 min. heating at 850°C. is sufficient to complete the fusion.

When fusion is complete, allow the crucible and contents to cool below 100°C., then add 2 or 3 ml of water and heat over a low flame, using care to prevent spattering, until the contents can be separated from the crucible. Transfer the fused mixture to a 150 ml beaker with the aid of about 25 ml of hot water. Boil until the caked material is completely disintegrated, then filter thru a retentive filter paper. Wash the residue on the filter with two 15 ml portions of hot 5% Na_2CO_3 soln. Lead will be in both filtrate and residue. Transfer the filtrate to a separatory funnel and proceed to extract the lead from the filtrate as directed under aluminum lakes. Dissolve the residue on the filter in 10–20 ml of the hydrochloric acid soln, wash the filter with water and add washings to the soln. Boil the soln to expel carbon dioxide, then transfer to a separatory funnel and extract the lead as directed above. Combine with the chloroform extracts from the soluble portion of the fusion products and determine the total lead by the electrolytic method. (6)

RESULTS

The method described was used to test the recovery of lead ranging from 40 micrograms to 200 micrograms added to 2 gram samples of D&C Red No. 9, Barium Lake. The average recovery was 98 per cent. Results are shown in Table 3.

DISCUSSION

The use of sodium nitrate in the fusion mixture serves a dual purpose; the lead is kept oxidized, thus minimizing the possibility of loss of lead through volatilization, and the fusion temperature is lowered as a result of its flux properties.

CONCLUSION

The method described above appears to give accurate and reproducible results.

TABLE 3.—*Recovery of lead*

BLANK* MICROGRAMS Pb	Pb ADDED	EQUIVALENT TO PER CENT	NET Pb RECOVERED		AVERAGE RECOVERY
	micrograms		*micrograms*	*per cent*	*per cent*
40	40	0.0020	37	93	97
			36	90	
			39	97	
			42	106	
			39	98	
41	80	0.0040	76	96	97
			79	98	
			73	91	
			80	100	
			79	98	
41	100	0.0050	101	101	99
			100	100	
			96	96	
			100	100	
			98	98	
41	120	0.0060	115	96	98
			121	100	
			118	98	
			118	98	
41	200	0.0100	201	100	99
			201	100	
			198	99	
			197	98	
			200	98	

* The blank includes the electrode blank, reagent blank, and lead originally present in the lake.

REFERENCES

(1) WICHMANN, H.J., *et al.*, *This Journal*, **17**, 108 (1934).
(2) CLIFFORD, PAUL, *Ibid.*, **26**, 26 (1943).
(3) ETTELSTEIN, N., *Ibid.*, **26**, 552 (1947).
(4) *Methods of Analysis*, A.O.A.C., 6th Ed., **29.40** (1945), p. 460.
(5) *Ibid.*, **29.36**, p. 456.
(6) *Ibid.*, **29.39**, **29.40**, **29.41**, pp. 459–461.
(7) KRESS, "Proceedings of the Scientific Section of the Toilet Goods Association, Inc.," 4–20 (1945).

DETERMINATION OF ROTENONE IN SMALL SAMPLES

By Merriam A. Jones and Caleb Pagán[1]

The rotenone content of an individual root or part of a root is often required in a breeding and selection program or a physiological study. In such instances the quantity of plant material available for analysis is frequently so small that the regular gravimetric[2] or titrimetric methods[3] cannot be used.

Two modifications of these methods have been devised for the determination of rotenone in small samples. Since the methods involve less than one-tenth the amount of sample required by the official macro methods, they might properly be termed micro methods.

In devising the procedure it was desired that the new methods should involve the same principles as the official method so that the results obtained would be directly comparable. This end was accomplished by using a micro beaker to which was sealed a spout containing a sintered glass filter. Small samples can be handled in this device without involving the numerous transfers required by the macro methods. The micro beaker shown in Figure 1 is an inexpensive stock item in catalogs of laboratory apparatus. The procedure was so arranged that, after the extraction of the sample, either a gravimetric or titrimetric method could be used.

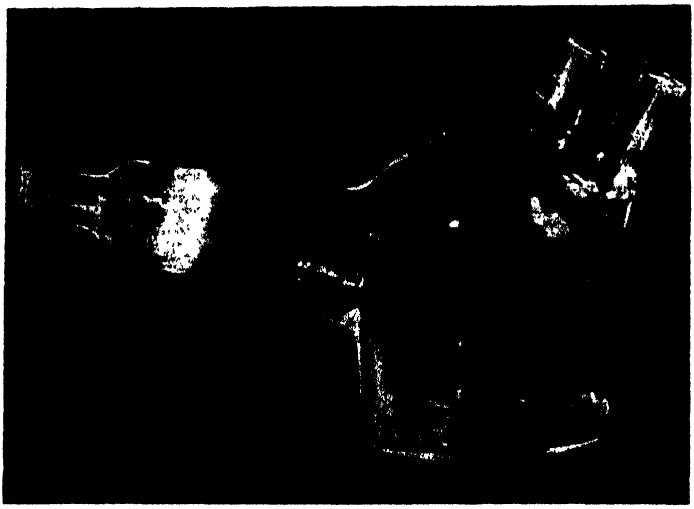

Fig. 1.—Micro beaker, for use in determination of small samples.

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[1] Former chemist and present chemist, respectively, Federal Experiment Station in Puerto Rico, Mayagües, P. R.

[2] A.O.A.C., *Official and Tentative Methods of Analysis*, Ed. 6 (1945).

[3] Jones, Howard A. "A Titrimetric Step in Determining Rotenone." *Ind. Eng. Chem., Anal. Ed.*, 10 684 (1938).

EXTRACTION OF SAMPLE

Weigh 2.000 gm of powdered root, ground to pass a 0.5 mm sieve and mix in a one-ounce screw-cap bottle with 0.66 gm of Norite-A decolorizing carbon. Add 20.0 ml of chloroform. Screw on the cap tightly and weigh to the nearest hundredth gram. (The inner surface of the cap of the screw-top bottle should be lined with cork or paper to prevent leakage and this material should be protected from the chloroform by aluminum foil.) Shake 4 hours, leave overnight, and shake 1 hour. Weigh the bottle, and replace any solvent lost by evaporation. Cool in ice bath for 30 min. and filter thru fast filter paper (11 cm). During filtration, cover the funnel with a beaker cover and plug the neck of the receiving flask with cotton to reduce loss by evaporation. Sample the filtrate after it has come to room temp.

GRAVIMETRIC PROCEDURE

Pipet 5.00 ml of the extract into a tared micro beaker (Fig. 1). Evaporate solvent on a steam bath while passing a slow current of air thru the beaker by means of suction on the filter spout. (The steam bath used consisted of a wide-mouth conical flask (250 ml), containing boiling chips or an anti-bump capillary.) Pipet another 5.00 ml of the extract into the beaker and evaporate as before. Add 2 ml of CCl_4 to the resinous residue, warm, and swirl. Evaporate solvent as before and repeat with another 2 ml of CCl_4. Cool and weigh to the nearest hundredth gram. (Weighings for the purpose of replacing evaporated solvents may be made on a torsion balance which allows for rapid weighing to .01 gram.) Multiply the weight of the residue in hundredths grams by 10 and add this number of ml of CCl_4 saturated with rotenone. Weigh beaker to .01 gram and warm to dissolve resin. When cool, reweigh, and replace with pure solvent any CCl_4 lost by evaporation. Cool in ice water and seed, if necessary, until solvate crystallizes. Keep at 0°C. for 4 hours or overnight. Filter by applying light suction to the spout. (For filtration an ordinary suction flask was used. A conical hole was cut in the rubber stopper so that the filter spout could be readily inserted and removed.) Add a volume of alcohol saturated with rotenone at room temp. equal to the volume of rotenone-saturated CCl_4 previously used. Weigh beaker to .01 gram, and warm to dissolve solvate in alcohol. Reweigh, and replace with pure solvent any alcohol lost by evaporation. Leave at room temp. 4 hours or overnight. Filter by gravity using a light suction to remove last of alcohol. Dry at 105° for 30 min., cool, and weigh to 0.1 mg. This weight minus the tare is multiplied by 100 to give the per cent rotenone in the root.

VOLUMETRIC PROCEDURE

Proceed as described under gravimetric procedure down to the point at which alcohol is added to the rotenone-CCl_4 complex. At this point, instead of adding alcohol, add 2 ml of acetone. Warm to dissolve precipitate in acetone, and evaporate acetone on steam bath. Add 0.5 ml of dichloroacetic acid and warm until soln is effected. Cool in ice-water for 2 min. while swirling. Add 10 drops of ice-water dropwise at the rate of 4 drops per min. while swirling the beaker. (The dropping pipet should consist of a glass tube drawn out so that about 40 drops equal 1 ml.) Seed with rotenone-dichloroacetic acid complex and add 5 drops of ice-water at 30-second intervals. Precipitation usually occurs within 5 min. If no precipitate forms, add ice-water dropwise at the rate of 4 drops per min., while swirling the beaker in the ice-water bath, until precipitation occurs. Swirl for several min. in the ice-water bath to complete the precipitation. Add 10 drops of ice-water at 10-sec. intervals while shaking. Add 10 drops rapidly while shaking, and then add 20 drops without shaking. Filter by tilting the beaker and applying gentle suction at the spout. Wash precipitate with 25 ml of water. Dissolve precipitate and wash out of device

with 5 ml of chloroform into a 50-ml beaker or conical flask. Add 10 ml of freshly boiled water, 3 drops 0.1 per cent phenolphthalein, and titrate with 0.01 *N* NaOH. Each ml of 0.1 *N* NaOH is equivalent to 3.94 mg of rotenone.

RESULTS AND DISCUSSION

The same precautions required in the official gravimetric method and its titrimetric modification should be observed in the micro methods. Two sources of possible error should be specially considered: (1) Incomplete crystallization of the solvate, and (2) loss of resin particles by entrainment when evaporating the chloroform. By following the method exactly as outlined crystallization of the CCl_4-rotenone complex should be obtained with minimum loss of resin by entrainment.

In the volumetric procedure it is essential that a crystalline rotenone-dichloroacetic acid precipitate be obtained. If an amorphous complex is obtained, excess acid will be retained by the precipitate introducing an

TABLE 1.—*Comparison of the results obtained by three methods in rotenone analysis of some derris and lonchocarpus root samples*

SAMPLE	ROTENONE		
	OFFICIAL MACRO	GRAVIMETRIC MICRO	VOLUMETRIC MICRO
	Per cent	*Per cent*	*Per cent*
Derris 1	1.8	1.85 1.81	1.90 1.82
Derris 2	4.2	4.17 4.12	4.21 4.32
Derris 3	4.7	4.62 4.79	4.76 4.80
Derris 4	5.6	5.60 5.58	5.68 5.66
Derris 5	5.9	5.82 6.01	5.86 5.82
Derris 6	7.2	7.19 7.25	7.17 7.23
Lonchocarpus 1	4.5	4.47 4.52	4.46 4.50
Lonchocarpus 2	5.6	5.59 5.55	5.63 5.61
Lonchocarpus 3	7.3	7.22 7.30	7.41 7.19

error of unknown magnitude. For comparison of the results of the micro methods with the official macro method, a series of derris and lonchocarpus samples were chosen of low, medium, and high rotenone content. The results of the analyses by the three methods are shown in Table 1.

SUMMARY

Methods are described for the analysis of small samples of rotenone by a gravimetric and by a titrimetric procedure. The results compare favorably with those obtained by the official macro method.

DENSITY AND REFRACTIVE INDICES OF LACTOSE SOLUTIONS

By EMMA J. McDONALD and ANNE L. TURCOTTE (National Bureau of Standards, Department of Commerce, Washington, D. C.)

I. INTRODUCTION

Lactose, the disaccharide commonly known as milk sugar, has for many years been of commercial importance. Until recently its principal use was in the manufacture of pharmaceuticals, food products, and in the preparation of modified diets; however, the penicillin industry now also requires large quantities of this sugar.[1]

In the present investigation measurements have been made to determine the densities and refractive indices of lactose solutions. The tables here presented are expected to be of use to the investigator working with the pure solutions, as well as to the analyst dealing with solutions in which the total solids may be calculated as lactose.

II. PREPARATION OF LACTOSE

The lactose used in this investigation was carefully prepared by repeated crystallizations of the commercial product. The procedure consisted in heating a 50-per cent solution in a water bath at 90°C., treating with decolorizing carbon, and filtering. The filtrate was then seeded with α-lactose hydrate crystals and stirred continuously while cooling in an ice bath. The resulting crystals, which were uniformly small and well formed, were purged on a centrifugal machine and washed consecutively with cold water and alcohol. After three crystallizations the ash content had decreased to .002 per cent and remained constant. The specific rotation of α-lactose hydrate in equilibrium solution is $+50.53°$. Thus the direct-reading of a 10 per cent solution would amount to $+30.35°$. The effect of the last traces of impurities on this reading is too slight to affect the accuracy. Analysis by the method of Munson and Walker also showed the product to be unchanged within the accuracy of the method. Here, as in the case of specific rotation, small traces of impurities would not affect the results noticeably. We, therefore, believe that ash content is a more reliable criterion for purity. The recrystallized product was dried in air and stored in a desiccator over commercial α-lactose hydrate. Under these conditions the vapor pressure was so maintained that loss of water of crystallization was prevented.

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[1] E. O. Whittier, *J. Dairy Science*, 27, 505 (1944); a review, "Lactose and Its Utilization," contains many references to work on lactose.

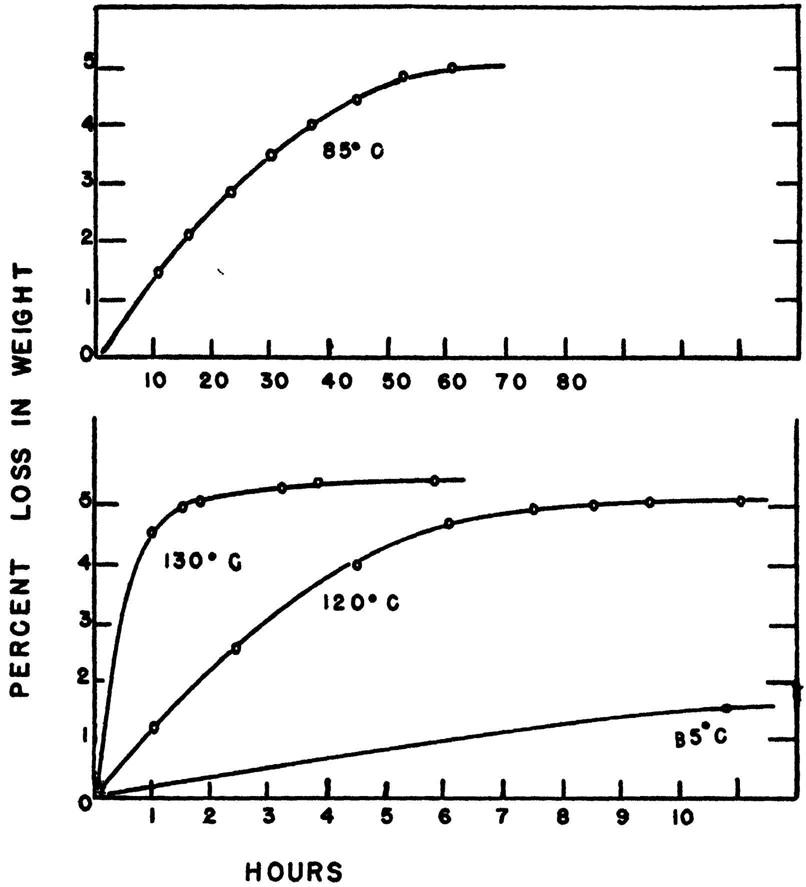

FIG. 1.—Dehydration of α-lactose hydrate at 85°, 120°, and 130°C. in vacuum.

Lactose hydrate contains one molecule of water of crystallization, which amounts to 5 per cent of the total weight. Any change in the water of hydration would be reflected as an error in the percentage composition of the prepared solutions, and hence in the reported physical constants; we, therefore, carefully investigated the composition of the crystals as to moisture content and were assured that we were dealing with a hydrate of uniform composition. Many moisture determinations were run in order to determine the most favorable conditions for this analysis.

The results obtained when the hydrate was dried in a vacuum at 85°, 120°, and 130°C. are shown in Figure 1. In all cases the loss in water was accompanied by some decomposition of the sugar as indicated by a yellowing of the sample. This discoloration was more pronounced in the recrystallized sugar than in the original commercial product. Because the ash content decreased on recrystallization from 0.0135 to 0.002 per cent, it is suggested that a buffering material is present in the commercial product and that this retards decomposition. The minimum discoloration during loss of water appeared to take place at 120°C., and, therefore, this temperature was subsequently used in determining the moisture content. The drying curves shown in Figure 2 are representative of those obtained during the progress of the investigation. Inspection of these curves shows the danger of using an arbitrary time when determining the moisture content of any given sample of α-lactose hydrate.

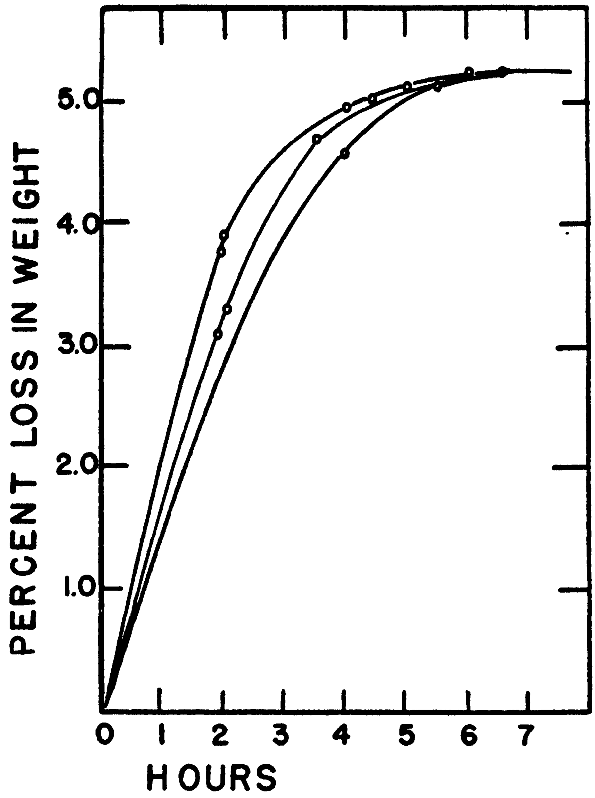

Fig. 2.—Dehydration of α-lactose hydrate at 120°C. in vacuum.

III. PHYSICAL MEASUREMENTS

The range in concentration where accurate density and refractive index measurements can be made is limited by the relatively low solubility of lactose and by the readiness with which supersaturated solutions tend to deposit crystals. At 20°C. a saturated solution of lactose contains 16 per cent of the sugar, a low value as compared with 49, 67, and 70, the respective percentages of saturated dextrose, sucrose, and levulose solutions at this temperature. Supersaturated solutions deposit lactose hydrate crystals with great ease, and thus limit the reliability of measurements made upon them. We have, therefore, carried out density and refractive index measurements with high precision in the range of unsaturation. The values reported for supersaturated solutions, although not obtained with the same precision, are suitable for many purposes and thus were included in this investigation.

1. *DENSITY OF LACTOSE SOLUTIONS*

Density measurements were made in a carefully calibrated flask. When filled to the lowest graduation at 20°C., this flask contained 126.333 ml. Its neck, 6 mm inside diameter, was graduated in 10 divisions at intervals of 0.02 ml. The volume reading could be estimated to one-tenth of one of these divisions, thus permitting a precision slightly greater than two units in the fifth place of density. The flask was recalibrated from time to time during the progress of the investigation, and the volume was found to be constant within the error of reading.

A quantity of α-lactose hydrate was introduced into the weighed flask and accurately weighed. The sugar was dissolved in distilled water, and air was removed from the solution by placing the slightly warmed flask in an environment of reduced pressure, care being taken to avoid spattering during this procedure. Water was then added to some point within the graduation, and the flask was rotated in such a way that the solution did not wet the upper part of the neck of the flask. After removing the water adhering to the flask above the solution by means of a current of dry air, the flask was placed in a thermostat and the final volume taken when the solution had come to temperature. Since the manipulation was time-consuming, mutarotation was completed before the final observation of volume.[2]

At the time of each weighing, the density of the air in the balance case was determined by weighing a glass bulb, whose true mass had been determined at this Bureau. All weights were converted to weight in vacuo, the value for the density of solid α-lactose hydrate being taken as 1.540 in accordance with the determinations recorded below.

The experimental data are assembled in Tables 1 and 2. From these ob-

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[2] The procedure used in making the density determinations was essentially that described by Jackson and Matthews in their work on levulose. *NBS Jour. Research*, **8**, 405 (1932) R P 426.

TABLE 1.—*Density of lactose solutions at 20.0°C.*

WEIGHT OF LACTOSE[1] (AIR BRASS WTS.)	WEIGHT OF LACTOSE (VACUUM)	WEIGHT OF SOLUTION (VACUUM)	VOLUME OF SOLUTION	CONCENTRATION OF LACTOSE	D_4^{20} OBSERVED	D_4^{20} CALCULATED BY FORMULA[2]	DEVIATION FROM FORMULA
g	*g*	*g*	*ml*	*per cent*			
2.2413	2.2427	126.9997	126.381	1.766	1.00490	1.00487	+.00003
2.7596	2.7613	127.1586	126.344	2.172	1.00644	1.00641	+.00003
5.8969	5.9004	128.3891	126.405	4.596	1.01570	1.01569	+.00001
10.9203	10.9268	130.2569	126.404	8.389	1.03048	1.03050	−.00002
15.8408	15.8502	132.1234	126.444	11.996	1.04492	1.04493	−.00001
17.1718	17.1903	132.6147	126.442	12.963	1.04882	1.04885	−.00003
23.5294	23.5441	134.9147	126.398	17.451	1.06738	1.06738	0
24.0298	24.0450	135.1012	126.402	17.798	1.06884	1.06884	0
25.3294	25.3444	135.6730	126.493	18.681	1.07257	1.07255	+.00002

[1] α-lactose hydrate.
[2] $D_4^{20} = .99823 + .003739p + .00001281p^2$.

TABLE 2.—*Density of lactose solutions at 25.0°C.*

WEIGHT OF LACTOSE[1] (AIR BRASS WTS.)	WEIGHT OF LACTOSE (VACUUM)	WEIGHT OF SOLUTION (VACUUM)	VOLUME OF SOLUTION	CONCENTRATION OF LACTOSE	D_4^{25} OBSERVED	D_4^{25} CALCULATED BY FORMULA[2]	DEVIATION FROM FORMULA
g	*g*	*g*	*ml*	*per cent*			
1.0643	1.0650	126.4207	126.390	0.843	1.00024	1.00021	+.00003
1.8585	1.8596	126.7549	126.432	1.467	1.00255	1.00255	0
5.2778	5.2810	128.0341	126.436	4.125	1.01264	1.01262	+.00002
8.1789	8.2838	129.1437	126.470	6.337	1.02114	1.02113	+.00001
10.2912	10.2974	129.9499	126.496	7.924	1.02730	1.02732	−.00002
15.1632	15.1723	131.7102	126.457	11.519	1.04154	1.04157	−.00003
20.1185	20.1306	133.5434	126.463	15.074	1.05599	1.05598	+.00001

[1] α-lactose hydrate.
[2] $D_4^{25} = .99707 + .0037175p + .00001263p^2$.

served values the following equations were calculated by the method of least squares. Here the density of a lactose solution is expressed as a function of the percentage (p), of α-lactose present.

$$D_4^{20} = .99823 + .003739p + .00001281p^2 \quad (1)$$

$$D_4^{25} = .99707 + .003717p + .00001263p^2 \quad (2)$$

The deviation of the observed values from those calculated by formula are given in the last column of each table. The observed values show a mean deviation of less than 3 in the fifth place at 20°C., and less than 2 at 25°C., from those calculated by formula. We, therefore, believe that the formulas are valid to a few units in the fifth decimal.

TABLE 3.—*Density of lactose solutions at 20° and 25°C.*

α LACTOSE HYDRATE	D_4^{20}[1]	D_4^{25}[2]	α LACTOSE HYDRATE	D_4^{20}[3]
per cent				
0	.99823	.99707	20	1.0782
1	1.00198	1.00080	22	1.0869
2	1.00576	1.00456	24	1.0956
3	1.00956	1.00834	26	1.1045
4	1.01339	1.01214	28	1.1136
5	1.01725	1.01597	30	1.1227
6	1.02113	1.01983	32	1.1320
7	1.02503	1.02371	34	1.1414
8	1.02896	1.02762	36	1.1509
9	1.03292	1.03155	38	1.1605
10	1.03690	1.03551	40	1.1702
11	1.04091	1.03949	42	1.1801
12	1.04494	1.04350	44	1.1900
13	1.04900	1.04753	46	1.2001
14	1.05309	1.05159	48	1.2104
15	1.05720	1.05568	50	1.2207
16	1.06133	1.05978		
17	1.06550	1.06392		
18	1.06968			

[1] $D_4^{20} = .99823 + .003739p + .00001281p^2$.

[2] $D_4^{25} = .99707 + .0037175p + .00001263p^2$.

[3] $D_4^{20} = .9982 + .00370p + .0000150p^2$.

The values now given in the literature for the density of lactose solutions have been determined in connection with specific rotation studies. The work of Schmoeger (1), as well as that of Fleischman and Weigner (2), will be considered because these investigators extended their observations to supersaturated solutions. Schmoeger reported values for the density of lactose solutions containing as much as 36 per cent of the sugar. His values are given to the fourth decimal place. Fleischman and Weigner obtained data on solutions containing as much as 69 per cent of lactose. The latter authors report that great difficulty was encountered in preparing solutions of the higher concentrations and hence only few observations were made. We believe that the inclusion of density values on supersaturated solutions, although they cannot be obtained with great precision, may be of value to investigators working with this sugar. Therefore, we have applied the method of least squares to the data presented by these early investigators on supersaturated solutions for concentrations as high as 50 per cent and obtained the following equation relating the density to the percentage concentration.

$$D_4^{20} = .9982 + .00370p + .0000150p^2 \tag{3}$$

Tables 3 and 4 are working tables containing density values for unsaturated solutions and for saturated solutions of concentrations up to 50 per cent. The values for unsaturated solutions are based on equations (1) and (2) while those for the supersaturated solutions were calculated by use of equation (3).

TABLE 4.—*Density of lactose solutions at 20°C.*

ANHYDROUS LACTOSE	D_4^{20}	ANHYDROUS LACTOSE	D_4^{20}
per cent		*per cent*	
1	1.00218	18	1.07367
2	1.00616	20	1.0827
3	1.01016	22	1.0919
4	1.01420	24	1.1012
5	1.01824	26	1.1107
6	1.02236	28	1.1203
7	1.02647	30	1.1300
8	1.03062	32	1.1399
9	1.03480	34	1.1498
10	1.03901	36	1.1600
11	1.04324	38	1.1702
12	1.04750	40	1.1806
13	1.05179	42	1.1911
14	1.05611	44	1.2017
15	1.06046	46	1.2125
16	1.06484	48	1.2234
17	1.06924	50	1.2345

2. *DENSITY OF CRYSTALLINE LACTOSE*

The density of the crystalline α-lactose hydrate[3] was required in order to convert the weight in air to weight in vacuum.

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[3] Previous values [3] Lichtenstein 1.543, Boedeker 1.5384, Filhol 1.534, Joule and Playfair 1.530, and Pionchon 1.525 [4] Hudson and Brown 1.54 (Sp. gr. α-lactose hydrate), 1.59 (sp. gr. β-lactose).

TABLE 5.—*Density of crystalline lactose*

WEIGHT PURE TOLUENE (VACUUM)	VOLUME TOLUENE	D_4^{20}[1] TOLUENE	WEIGHT α-LACTOSE HYDRATE (VACUUM)	WEIGHT TOLUENE (VACUUM)	TOTAL VOLUME	VOLUME TOLUENE	VOLUME α-LACTOSE HYDRATE	D_4^{20} α-LACTOSE HYDRATE
g	*ml*		*g*	*g*	*ml*	*ml*	*ml*	
86.6441	100.2355	0.86441	19.1054	98.5649	126.4265	114.0243	12.4022	1.5405
86.5196	100.0915	0.86441	19.1054	98.6121	126.4838	114.0789	12.4049	1.5401
86.6413	100.2285	0.86444	19.1054	98.5252	126.3883	113.9784	12.4099	1.5395
86.5947	100.1765	0.86442	20.0613	97.9627	126.3597	113.3277	13.0320	1.5394
86.5650	100.1425	0.86442						
	average	0.86442						
86.6176	100.1685	0.86472	10.2736	103.5757	126.4532	119.7836	6.6696	1.5404
86.6241	100.1805	0.86468	10.2736	103.5025	126.3692	119.6989	6.6703	1.5402
86.5739	100.1235	0.86467						
	average	0.86469					average	1.5400
			WEIGHT β-LACTOSE (VACUUM)				VOLUME β-LACTOSE	D_4^{20} β-LACTOSE
			g				ml	
		0.86469	22.8752	96.8837	126.4704	112.0794	14.3910	1.5895
		0.86469	22.8752	96.7750	126.3501	111.9537	14.3964	1.5890
		0.86469	22.8752	96.8459	126.4265	112.0357	14.3908	1.5896
							average	1.5893

[1] Samples of toluene from two different lots were used for these experiments. The density of toluene recorded in the determinations on β-lactose is the average value for the second lot of reagent.

The lactose was weighed in a calibrated flask, and the flask was filled with dry toluene saturated with lactose at 20°C. The trapped air was removed from the crystals by applying gentle suction while rotating the flask. All weights were converted to weight in vacuo. The results of these measurements are given in Table 5. The density of lactose hydrate was found to be 1.540. The density of commercial β-lactose, an anhydrous form that has found extensive commercial use because of its greater solubility, was found to be 1.589.

3. *REFRACTIVE INDICES*

The refractive indices of unsaturated solutions of lactose at 20°C. and 25°C. were determined by use of a carefully calibrated immersion refractometer under accurately controlled conditions of temperature. All measurements were made in a constant temperature room whose temperature was maintained within two degrees of that at which the water in the bath surrounding the instrument was held.

The instrument was inserted in a large glass tube, 83 mm in diameter and 350 mm in length. This contained sufficient water to surround the instrument cup containing the sugar solution. The glass tube with the suspended instrument was placed in a constant temperature water bath so that only a small part of it extended above the surface of the water. A thermometer was placed beside the instrument. Illumination was supplied by an electric bulb submerged in a glass tube similar to that containing the refractometer. The floor of the bath reflected the light into the instrument. Under these conditions a very sharp line characteristic of total refraction was observed, permitting a precision, and an estimated accuracy, of 0.1 to 0.2 of a scale division in the reading of the instrument.

The conversion tables supplied by the manufacturer are based on readings for distilled water. In accord with these tables the instrument was set at 14.50 at 20°C. and at 13.25 at 25°C. These readings are equivalent to indices of 1.33299 at 20°C. and 1.33252 at 25°C. Readings on distilled water were made frequently, thus insuring the setting of the instrument. The arbitrary scale readings were converted to refractive indices by use of the manufacturer's tables.

Equations relating the refractive index to the concentration were prepared by subjecting the observed data to the method of least squares.

$$n_D^{20} = 1.33299 + .001409p + .00000498p^2 \tag{4}$$

$$n_D^{25} = 1.33251 + .001405p + .000004805p^2 \tag{5}$$

Tables 6 and 7 contain the observed data as well as those calculated by formula.

The refractive indices of supersaturated solutions at 25°C., as well as those of all concentrations at 15°C., were determined with an Abbé refractometer. The scale of the instrument used permitted readings to a few

TABLE 6.—*Refractive indices of lactose solutions at 20.0°C.*

WEIGHT OF LACTOSE[1] (AIR BRASS WTS.)	WEIGHT OF LACTOSE (VACUUM)	WEIGHT OF SOLUTION (VACUUM)	CONCENTRATION OF LACTOSE	ZEISS IMMERSION SCALE READING t=20.0°C.	n_D^{20} OBSERVED	n_D^{20} CALCULATED BY FORMULA[2]	DEVIATION FROM FORMULA
g	*g*	*g*	*per cent*				
2.2413	2.2427	126.9997	1.766	21.00	1.33551	1.33549	+.00002
3.3207	3.3227	127.3975	2.608	24.12	1.33671	1.33669	+.00002
5.2365	5.2397	101.7278	5.151	33.74	1.34038	1.34038	0
7.3119	7.3162	102.5546	7.134	41.47	1.34330	1.34329	+.00001
15.1730	15.1819	131.8051	11.518	59.10	1.34988	1.34988	0
15.2437	15.2525	131.8654	11.567	59.30	1.34995	1.34996	−.00001
22.2235	22.2367	134.4964	16.533	80.41	1.35765	1.35764	+.00001

[1] α-lactose hydrate.

[2] $n_D^{20}=1.33299+.001409p+.00000498p^2$.

TABLE 7.—*Refractive indices of lactose solutions at 25.0°C.*

WEIGHT OF LACTOSE[1] (AIR BRASS WTS.)	WEIGHT OF LACTOSE (VACUUM)	WEIGHT OF SOLUTION (VACUUM)	CONCENTRATION OF LACTOSE	ZEISS IMMERSION SCALE READING to 25.0°C.	n_D^{25} OBSERVED	n_D^{25} CALCULATED BY FORMULA[2]	DEVIATION FROM FORMULA
g	*g*	*g*	*per cent*				
1.0643	1.0650	126.4207	0.843	16.32	1.33371	1.33370	+.00001
2.5473	2.5498	127.0832	2.006	20.54	1.33534	1.33535	−.00001
5.2778	5.2810	128.0341	4.125	28.54	1.33841	1.33839	+.00002
8.1789	8.1838	129.1437	6.337	36.90	1.34158	1.34160	−.00002
10.2912	10.2974	129.9499	7.924	43.20	1.34396	1.34395	+.00001
15.1632	15.1723	131.7102	11.519	57.63	1.34933	1.34933	0
20.1185	20.1306	133.5434	15.074	72.46	1.35478	1.35478	0

[1] α-lactose hydrate.

[2] $n_D^{25}=1.33251+.001405p+.000004805p^2$.

TABLE 8.—*Refractive indices of lactose solutions at 25°C.*

WEIGHT OF LACTOSE[1] (AIR BRASS WTS.)	WEIGHT OF LACTOSE (VACUUM)	WEIGHT OF SOLUTION (VACUUM)	CONCENTRATION OF LACTOSE	n_D^{25} OBSERVED ABBÉ	n_D^{25} CALCULATED BY FORMULA[2]	DEVIATION FROM FORMULA
g	*g*	*g*	*per cent*			
10.9856	10.9923	53.7058	20.468	1.3634	1.3634	.0000
14.0010	14.0095	54.9307	25.504	1.3720	1.3719	+.0001
17.1285	17.1389	56.6435	30.257	1.3800	1.3800	.0000
20.2416	20.3139	56.8220	35.750	1.3899	1.3900	−.0001

[1] α-lactose hydrate.
[2] $n_D^{25}=1.3325+.001384p+.00000624p^2$.

TABLE 9.—*Refractive indices of lactose solutions at 15°C.*

WEIGHT OF LACTOSE[1] (AIR BRASS WTS.)	WEIGHT OF LACTOSE (VACUUM)	WEIGHT OF SOLUTION (VACUUM)	CONCENTRATION OF LACTOSE	n_D^{15} OBSERVED ABBÉ	n_D^{1} CALCULATED BY FORMULA[2]	DEVIATION FROM FORMULA
g	*g*	*g*	*per cent*			
2.6839	2.6855	50.7500	5.292	1.3410	1.3410	.0000
13.1331	13.1412	131.1152	10.023	1.3480	1.3480	.0000
26.3086	26.3248	136.0419	19.351	1.3629	1.3627	+.0002
33.1227	33.1431	138.4840	23.933	1.3701	1.3703	−.0002
39.6613	39.6857	141.0027	28.145	1.3772	1.3774	−.0002
52.8430	52.8755	145.7118	36.288	1.3919	1.3917	+.0002

[1] α-lactose hydrate
[2] $n_D^{15}=1.3334+.001412p+.00000537p^2$.

units in the fourth decimal place. This was the limiting factor in the precision of the readings since they were all made under carefully controlled conditions. Equations 6 and 7 are based upon these data.

$$n_D^{25} = 1.3325 + .001384p + .00000624p^2 \tag{6}$$

$$n_D^{15} = 1.3334 + .001412p + .00000537p^2. \tag{7}$$

The observed data for indices of refraction, and also the values calculated by the formulas, are given in Tables 8 and 9. Table 10 is included as a working table. It has been prepared by use of the proper formula for each temperature.

TABLE 10.—*Refractive indices of lactose solutions*

LACTOSE HYDRATE	n_D^{25}	n_D^{20}	n_D^{15}	$\Delta n/\Delta t$
per cent				
1	1.33392	1.33440	1.3348	.000096
2	1.33534	1.33583	1.3362	.000098
3	1.33677	1.33726	1.3376	.000098
4	1.33821	1.33871	1.3391	.000100
5	1.33966	1.34016	1.3406	.000100
6	1.34111	1.34162	1.3421	.000102
7	1.34258	1.34310	1.3436	.000104
8	1.34406	1.34458	1.3451	.000104
9	1.34554	1.34607	1.3466	.000106
10	1.34704	1.34758	1.3481	.000108
11	1.34855	1.34909	1.3496	.000108
12	1.35006	1.35062	1.3511	.000112
13	1.35159	1.35215	1.3526	.000112
14	1.35312	1.35369	1.3542	.000116
15	1.35467	1.35524	1.3558	.000116
16	1.35622	1.35681	1.3573	.000118
18	1.3594		1.3605	.00011
20	1.3627		1.3637	.00010
22	1.3660		1.3670	.00010
24	1.3693		1.3703	.00010
26	1.3727		1.3737	.00010
28	1.3762		1.3770	.00008
30	1.3797		1.3805	.00008
32	1.3832		1.3839	.00007
34	1.3868		1.3875	.00007
36	1.3904		1.3910	.00006

IV. SUMMARY

Density and refractive index values are reported for lactose solutions over the range of unsaturation with a precision of 3 or 4 in the fifth decimal place at 20°C. and at 25°C. For solutions of higher concentrations, up to 50

per cent, the corresponding values are reported at 25°C., and refractive indices alone at 15° C.

REFERENCES CITED

(1) M. Schmoeger, *Ber. deut. chem. Ges.*, **13**, 1922 (1880).

(2) W. Fleischmann and G. Weigner, *J. Landw.*, **58**, 45–64 (1910).

(3) E. O. von Lippmann, *Chemie der Zuckerarten*, p. 1526, von Friedrick Vieweg und Sohn (1904).

(4) C. S. Hudson and F. C. Brown, *J. Am. Chem. Soc.*, **30**, 960 (1908).

BOOK REVIEW

Dynamic Aspects of Biochemistry. By Ernest Baldwin. xviii+457 pages. The Macmillan Co., 60 Fifth Ave., New York, N. Y., 1947. Price $4.00.

The chemical reactions accompanying biological processes are rapidly becoming better understood; the individual steps which make up the mechanism of each are being discovered at an increasing pace; the chemical agents so characteristic and necessary for these life processes are assigned the name enzymes. In this book, "Dynamic Aspects of Biochemistry," some of the various threads, which, when woven together make up a living organism, are systematized to the extent that present knowledge allows.

This book is designed to cover a phase of biochemistry in a form suitable for textbook use in an advanced college course. The author is an experienced teacher and has used some innovations, particularly in writing equations, which give "a distinctly pictorial representation of chemical events." The book is divided into two parts: Part I. Enzymes; Part II. Metabolism. In Part I, after an introduction on "The General Behaviour and Properties of Enzymes" and on "The Nature of the Catalytic Process," some individual enzymes are discussed briefly by types as follows: (1) Hydrolases and Phosphorylases: Included in these are proteases, carbohydrases, lipases, and esterases. (2) Oxidizing Enzymes: Among these are certain well known oxidases, including catalases, the cytochrome oxidases, and those enzymes which oxidize compounds of the phenolic type. The dehydrogenases are also classified as oxidizing enzymes. (3) Other Enzymes: Adding, Transferring, and Isomerizing Enzymes. The individual enzymes are discussed briefly along with the fundamental reactions which each catalyzes.

In Part II, after a general discussion of "Methods Employed in the Investigation of Intermediary Metabolism" and "Food, Digestion, and Absorption," the metabolism of nitrogenous compounds, especially the proteins and amino acids, is described at length. Then follows a similar treatment of carbohydrates including both the aerobic and anaerobic systems. Finally the enzymatic transformations concerned with the metabolism of fats are discussed.

At the time when many regulatory chemists were students, so little was understood about the intricate and still complex mechanisms of food metabolism and of biological reactions in general, that the subject, if mentioned at all, was beclouded with mystery. To one who reads this book, much of this mystery is resolved into a series of definite chemical reactions in which both inorganic and organic chemistry play their parts. The problems of life processes are so complex that only a beginning toward their complete elucidation is made in this book. However, as in so many other cases, once the foundations are laid and the plan becomes more legible, then progress on the building as a whole becomes much more rapid. For those who would like to know more about the chemical dynamics of life processes this book can be recommended. It is particularly free of errors, and the style is much more readily understandable than one might expect in such a complex field.

W. I. Patterson

REPORT ON RIBOFLAVIN*

(FLUOROMETRIC AND MICROBIOLOGICAL METHODS)

By Henry W. Loy, Jr. (Food and Drug Administration, Federal Security Agency, Washington, D. C.),
Associate Referee

At the meeting of the Association last year, it was recommended that the fluorometric method for the assay for riboflavin, as described at that time,[1] be studied collaboratively during the coming year. In this method, which combines features of those described by Najjar,[2] Arnold,[3] and Rubin, *et al*,[4] the riboflavin is first extracted from the sample with dilute hydrochloric acid and a clear filtrate prepared in a manner similar to that used in the tentative microbiological method.[5] The final sample solution is acidified with acetic acid and treated with potassium permanganate and hydrogen peroxide. The riboflavin content is then calculated from the degree of fluorescence of the sample solution, of the sample solution plus an added amount of riboflavin, and of these solutions after reduction of the riboflavin with sodium hydrosulfite.

The collaborators in the fluorometric study were asked to assay two samples, No. 1, enriched flour, and No. 2, dried brewers yeast, by the specified fluorometric method, other fluorometric methods in which they might be interested, and the tentative microbiological method.

Fifteen collaborators, including the Associate Referee, submitted results obtained by the collaborative fluorometric method. Seven of these laboratories submitted results by other fluorometric methods and nine by the tentative microbiological method.

Comparisons between the averages of the results submitted for the different methods are shown in Table 1. Approximately 60 per cent of the values reported for each method were within a range of ±10 per cent of the average value obtained by the respective method. However, there was a wide divergence of some of the results reported for each method. Although many laboratories obtained acceptable results with any one method, there appeared to be no obvious explanation, except in two cases, for this wide divergence of some of the results. Therefore, the study did not lead to the recommendation of a fluorometric method for adoption this year, but it is expected that further studies will prove profitable.

Although good results may be obtained with the present tentative microbiological method,[5] it was believed by some collaborators that further consideration should be given to improvements that may be made in this method. Accordingly, those collaborators who were able to undertake additional work were asked to study a method that is similar to the present tentative microbiological method except for modifications in the preparation of the sample solution and of the photolyzed peptone and yeast supplement solutions used in the basal medium. Also, modifications were suggested for the handling of the culture and for the size of test tubes used in the assay.

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* This report was presented on Tuesday, October 21, 1947, at the last annual meeting and should have been printed with other reports on Vitamins in the August (1948) Journal.

[1] *This Journal*, 30, 392 (1947).

[2] *J. Biol. Chem.*, 141, 355 (1941).

[3] *Cereal Chemistry*, 22, 455 (1945).

[4] *Ind. Eng. Chem., Anal. Ed.*, 17, 136 (1945).

[5] *This Journal*, 30, 79 (1947).

TABLE 1.—*Average potency in riboflavin*

(Comparison of results obtained with the different methods used by the 15 reporting laboratories)

SAMPLE NO. 1—ENRICHED FLOUR
(*Mg. of riboflavin per lb. of sample*)

NO. OF COLLABORATORS REPORTING RESULTS	FLUOROMETRIC METHODS		MICROBIOLOGICAL METHODS		
	COLLABORATIVE	OTHER	TENTATIVE	COLLABORATIVE	OTHER
15	1.51				
7	1.53	1.37			
9	1.54		1.46		
4	1.41		1.42	1.34	
5	1.47		1.45		1.48

SAMPLE NO. 2—DRIED BREWERS YEAST
(*Mg. of riboflavin per g. of sample*)

NO. OF COLLABORATORS REPORTING RESULTS	FLUOROMETRIC METHODS		MICROBIOLOGICAL METHODS		
	COLLABORATIVE	OTHER	TENTATIVE	COLLABORATIVE	OTHER
15	0.043				
7	0.047	0.045			
9	0.043		0.042		
4	0.041		0.042	0.037	
5	0.038		0.040		0.037

The collaborators in the microbiological study were asked to assay the same two samples that were used in the fluorometric study. Four of the laboratories submitted results obtained by the tentative and collaborative microbiological methods, and five by the tentative and other microbiological methods.

Comparisons between the averages of the results submitted for the different methods used in the microbiological study also are shown in Table 1. Closer agreement in the results between the laboratories was obtained with the collaborative microbiological method than with the tentative microbiological method, but, since only four laboratories submitted comparative results, the data were considered to be insufficient to lead to the recommendation of any changes to be made in the tentative method. Except for the collaborative microbiological method, the range of values reported for each method was about the same as the range of values reported in the fluorometric study. Again, there appeared to be no obvious explanation for the wide divergence of some of the results. Although the microbiological study did not lead to the recommendation of any changes in the present tentative method, it is believed that further studies may do so.

COLLABORATORS

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The Associate Referee wishes to express appreciation to the collaborators and to their organizations for their cooperation in this study.

RECOMMENDATIONS*

It is recommended—

(1) That further work be conducted on the fluorometric method that was the subject of this year's study.

(2) That further consideration be given to improvements that may be made in the present microbiological method.

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* For report of Subcommittee A and action of the Association see *This Journal*, 30, 44 (1948).

CONTRIBUTED PAPERS

COMPARISON OF METHODS FOR THE DETERMINATION OF CAROTENE

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In the course of determining the carotene contents of various plant tissues commonly utilized as browse by forest animals, it was observed that widely divergent results were obtained when the analytical procedures were varied according to some of the commonly accepted methods. In order to establish the most suitable procedure, a series of carefully controlled experiments, involving the analysis of replicate samples by different methods, was conducted.

At least three fundamentally distinct procedures have been advanced for the extraction of carotenoid pigments from plant materials. One of these, as exemplified by the methods of Wall and Kelley (1) and Moore and Ely (2) utilizes a Waring Blendor or similar device to allow extraction by cold solvents. A second procedure, such as that prescribed in *Changes in Methods of Analysis* (3), depends upon a hot extraction with low-boiling hydrocarbons, or a mixture of hydrocarbons and a polar solvent. The third method involves a preliminary digestion of the plant material with hot alcoholic potash, followed by extraction with petroleum ether or other solvents. These three procedures were followed in the present series of experiments.

PROCEDURE

A. Preparation of Sample: A quantity of dehydrated alfalfa leaf meal, obtained from commercial sources, was ground to pass a 40-mesh screen, placed in a closed container, and stored in the refrigerator. Portions for analysis were removed and weighed immediately prior to assay. At least five analytical samples were examined by each procedure in the study.

B. Extraction of Pigments: The following procedures were used for the extraction of carotenoid pigments:

1. Method of Wall and Kelley (1): A 2–5 gram sample of the alfalfa leaf meal was extracted with 200 ml. of a 5:3 mixture of 95% ethyl alcohol and petroleum ether (B.P.—35–65°C.) in a Waring Blendor for 10 minutes. The extract and finely divided plant material were transferred to a fritted glass filter connected to a suction flask, and washed with alternate portions of alcohol and petroleum ether until a colorless filtrate was obtained. The alcohol was removed from the extracts by washing with water in a separatory funnel, and the petroleum ether extracts were concentrated on the steam bath, dried by filtration through anhydrous sodium

sulfate into a 100 ml. volumetric flask, and adjusted to volume with petroleum ether.

2. Modified A.O.A.C. Procedure (3): A 2–4 gram sample was extracted in a Bailey-Walker apparatus for one hour with 30 ml. of a 30% solution of acetone in petroleum ether. The extracts were transferred to a 100 ml. volumetric flask, and made to volume with petroleum ether.

3. Alcohol Potash Digestion: A 2–5 gram portion of the alfalfa leaf meal was placed in an Erlenmeyer flask with 100 ml. of 12% alcoholic potash, and digested on the steam bath for 30 minutes. The volume was held constant throughout the digestion by the addition of 95% alcohol. The digestion mixture was cooled to room temperature, transferred to a 500 ml. separator funnel, and the flask rinsed with 100 ml. of distilled water. The rinsings were added to the digestion mixture in the funnel, and the resulting solution extracted with 50 ml. portions of petroleum ether until a colorless extract was obtained (5 or 6 extractions). The extracts were combined in another separator, and 200 ml. distilled water added, care being taken not to shake or swirl the funnel during the process. After two minutes the aqueous layer was discarded, a second portion of 100 ml. water added, and the funnel swirled gently. After separation of the layers (2 minutes), the aqueous layer was removed, 25 ml. of water added, and the funnel shaken vigorously for 30 seconds. Additional extractions with 25 ml. portions of water were continued until the aqueous extracts were neutral to phenophthalein. After the final extraction, the funnel was allowed to stand for five minutes, any residual water removed, and the petroleum ether extracts concentrated to approximately 25 ml. under vacuum on the steam bath. The extracts were then filtered through anhydrous sodium sulfate into a 100 ml. volumetric flask, and made to volume with petroleum ether.

4. Saponification of Extracts: In order to determine the effect of saponification, an aliquot of the solution from (2) was taken, the solvent removed under nitrogen on the steam bath, and the residue treated as in (3).

C. Chromatography: All extracts were chromatographed on a column composed of a 1:3 mixture of magnesium oxide (Westvaco #2641) and Hyflo-Super Cel (Fisher). The column was prepared under suction in an absorption tube measuring 22×100 mm. fitted with a fritted disc of medium porosity. The adsorbent was packed to a depth of approximately 75 mm., and a 1 cm. layer of anhydrous sodium sulfate added. Following the addition of the extract solution, the chromatogram was developed, and carotene eluted, with a 5% solution of acetone in petroleum ether. The eluates were adjusted to desired volumes with petroleum ether.

D. Optical Measurements: The optical densities of the purified pigments were determined at 5 mμ intervals between 400 and 500 mμ, using a Beckman spectrophotometer.

DISCUSSION

When the extinction coefficients ($E^{1\%}_{1cm}$) of the carotene solutions were plotted against wave length (Fig. 1), it was apparent that the results obtained by the four procedures differed both qualitatively and quantitatively. All of the curves show maxima in the neighborhood of 450 and 475

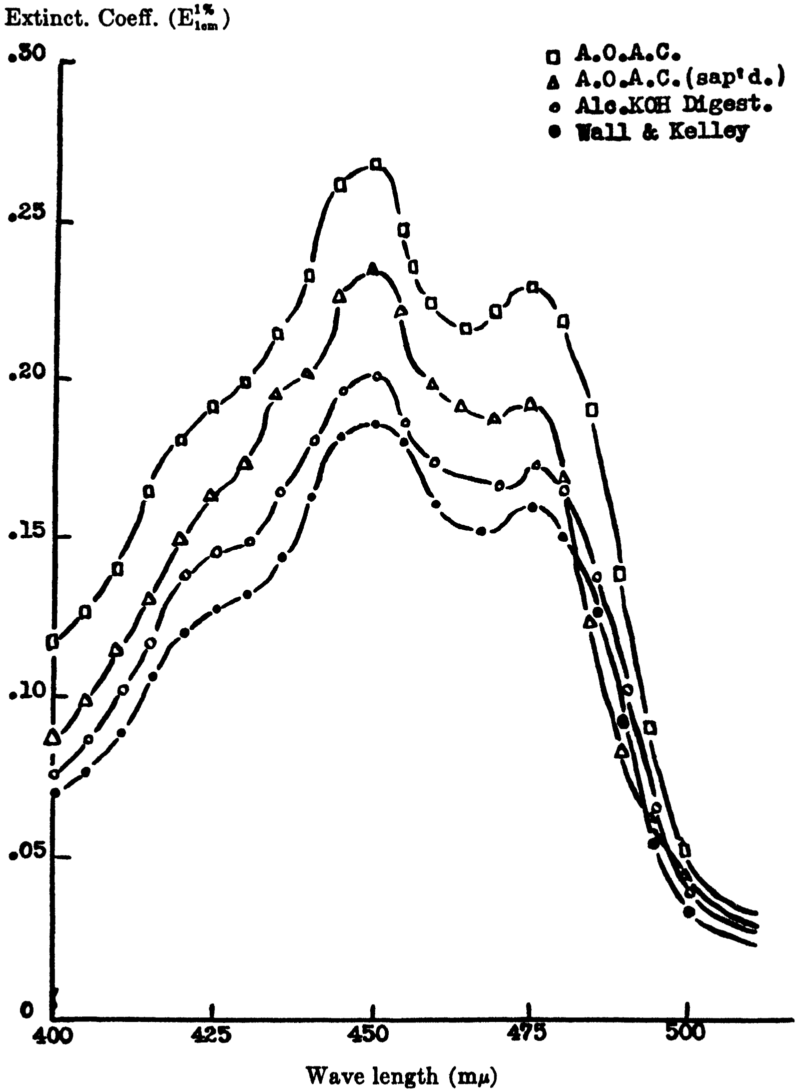

FIG. 1.—Extinction Coefficients of Carotene Solutions Obtained by Different Procedures (Averages for 5 assays by each method)

millimicrons, and in this respect, are typical for solutions of beta-carotene. However, marked differences in the shapes of the curves may be noted at points of secondary inflection, such as 420, 435, and 465 mμ, indicating that the extracts differed in the amounts of other carotenoid pigments. If the ratios E_{430}/E_{450}, E_{466}/E_{450}, and E_{478}/E_{450}, are calculated (Table 1), it will be seen that these curves correspond to that given by Harper and Zscheile (4) for pure beta-carotene. On the basis of these ratios, the solution obtained by the Wall and Kelley procedure would appear to contain the highest percentage of pure beta-carotene, although its total content of carotenoids is lower than that of any of the other methods. However, the degree of significance which may be attached to these variations in the values of these ratios was not determined, and it might be assumed that the pigment determined by each procedure was essentially beta-carotene.

TABLE 1.—*Ratios between extinction coefficients at different wave lengths*[1]

PROCEDURE	$\frac{E_{430}}{E_{450}}$	$\frac{E_{466}}{E_{450}}$	$\frac{E_{478}}{E_{450}}$
Mod. A.O.A.C.	0.755	0.81	0.83
Mod. A.O.A.C. (sap'd)	0.725	0.76	0.725
Alcoholic KOH Digestion	0.740	0.845	0.845
Wall & Kelley	0.705	0.81	0.81
(Theoretical)[2]	0.71	0.80	0.875

[1] Based upon averages of the results of five determinations by each method.
[2] *Cf.* Harper and Zscheile (4).

TABLE 2.—*Carotene determined in dehydrated alfalfa leaf meal by different methods*[1]

PROCEDURE	CAROTENE (MICROGRAMS/GRAMS)	
	450 mμ[2]	436 mμ[3]
Mod. A.O.A.C.	109.25	108.16
Mod. A.O.A.C. (sap'd)	95.47	98.98
Alcoholic KOH Digestion	80.29	82.91
Wall and Kelley	75.07	72.86

[1] Averages of the results of five determinations by each method.
[2] Calculated by the procedure in *Methods of Analysis* (5).
[3] Calculated by the procedure given in (3).

As shown in Figure 1, the extinction coefficient at 450 mμ for the solution from the modified A.O.A.C. procedure is 43 per cent higher than that for the Wall and Kelley method; 34 per cent higher than that for the alcoholic potash method; and 13 per cent higher than that for the saponified extracts. (This apparent superiority of the modified A.O.A.C. is shown in Table 2, where the carotene contents of the solution were calculated by two different procedures.) It will be noted that the amount of carotene found (based on the calculation at 450 mμ), by the modified A.O.A.C. procedure is 45 per cent greater than that found by the Wall and Kelley method. Since, in the Wall and Kelley method and the saponification methods, the procedure for the chromatographic separation of carotenes from other pigments, and for the subsequent spectrophotometric estimation, was the same, it appears that the observed differences in the results of assays on replicate samples were due to varying degrees of efficacy of the extraction technics.

SUMMARY

Replicate samples of dehydrated alfalfa leaf meal were assayed for carotene content by four different analytical procedures. The results obtained by the modified A.O.A.C. method were significantly higher than those obtained by the other procedures.

REFERENCES

(1) Wall, M. E., and Kelley, E. G., *Ind. Eng. Chem., Anal. Ed.*, 15, 18 (1943).
(2) Moore, L. A., and Ely, R., *Ibid.*, 13, 600 (1941).
(3) "Changes in Methods of Analysis," *This Journal*, 31, 111 (1948).
(4) Harper, R. H., and Zscheile, F. P., *Food Res.*, 10, 84 (1945).
(5) *Methods of Analysis, A.O.A.C.*, 6th Ed. (1945) 36.11, p. 604.

A STUDY OF METHODS FOR THE DETERMINATION OF SUGAR IN CROP PLANTS*

By Dorothy R. Waldron, C. D. Ball, E. J. Miller, and Erwin J. Benne (Michigan State College of Agriculture and Applied Science, East Lansing, Michigan)

For a number of years the authors have been concerned with the determination of sugars in a diversified variety of crop plants, including carrot and parsnip roots, onion bulbs, potato tubers, apple, cherry, pear, blueberry and tomato fruits, fruit tree leaves, and various kinds of grain and forage crops. Because of the many problems encountered in sampling, reducing to small pieces and mixing such materials, in order to obtain representative portions for analysis, and in extracting, isolating, and evaluating the sugar contained in them, an investigation was undertaken to test the efficiency and reliability of certain practices when applied to the determination of sugars in different types of plant tissues. Carrot roots, onion bulbs, spinach leaves, tomato fruits, and young brome grass were selected as representative crop types, and comparative values for total (reducing plus hydrolyzable) sugar in samples of each were obtained by use of the combinations described below of (a) two methods for extracting the sugars from the tissue, (b) two different extracting solvents, (c) two methods of hydrolysis, and (d) four methods for evaluating the amounts of sugar. An account of the investigation and the results obtained are presented in this paper.

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* A joint contribution from the Department of Agricultural Chemistry and the Kedzie Chemical Laboratory. Published with permission of the Director of the Agricultural Experiment Station as journal article no. 946 (n.s.).

EXPERIMENTAL

The determination of sugar in a given crop involved six general steps. A brief description of the performance of each step follows:

1. Selecting the sample from the field.—The first step in the analysis of each crop was to select from the field a sample of sufficient size to insure that it represented the average composition of the entire crop. Amounts selected for this purpose were necessarily governed by the extent, uniformity, and nature of the crop concerned.

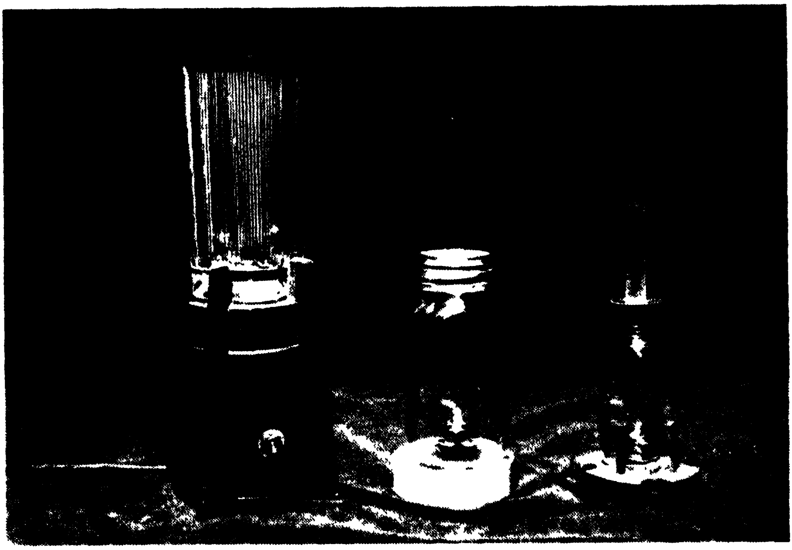

FIG. 1.—Special vessels devised for use with a Waring Blendor.

2. Reducing to small pieces and mixing the field sample.—

(a) Carrot roots and onion bulbs—Ground with food chopper and mixed by hand.

(b) Spinach leaves and young brome grass—Cut as finely as possible with hand shears and mixed by hand.

(c) Tomato fruits—All fruits in the entire field sample were cut in halves and thin transverse sections were cut from one half of each fruit for each analytical portion. Obviously no mixing was necessary with this practice.

3. Weighing the portions for analysis.—The balance used was sensitive to 0.05 gm. The weights of portions used for analysis varied with the sugar content of the tissue as indicated by preliminary experiments. In general they were comparable to those given below:

(a) Carrot roots	15 gms.
(b) Onion bulbs and tomato fruit	20 gms.
(c) Spinach leaves and young brome grass	15-25 gms.

4. Extracting the sugar from the tissue.—

(a) A Waring Blendor equipped with special vessels (Fig. 1), which were prepared in the authors' laboratory (1, 2), was used for comminuting the weighed portions of each tissue in the presence of the extracting solvent. The smaller vessel pictured was used with carrot roots, onion bulbs and tomato fruits; the quart fruit jar with the more bulky samples of spinach leaves and brome grass. Small amounts of calcium carbonate were added to the samples before they were blended, to neutralize the plant acids. The blending time was 4-9 minutes depending upon the nature of the tissue. Distilled water was used as the extracting solvent in one series of determinations and approximately 80% ethanol in another. In the latter series the amount of water present in the fresh tissue was taken into consideration in diluting 95% ethanol to the desired concentration.

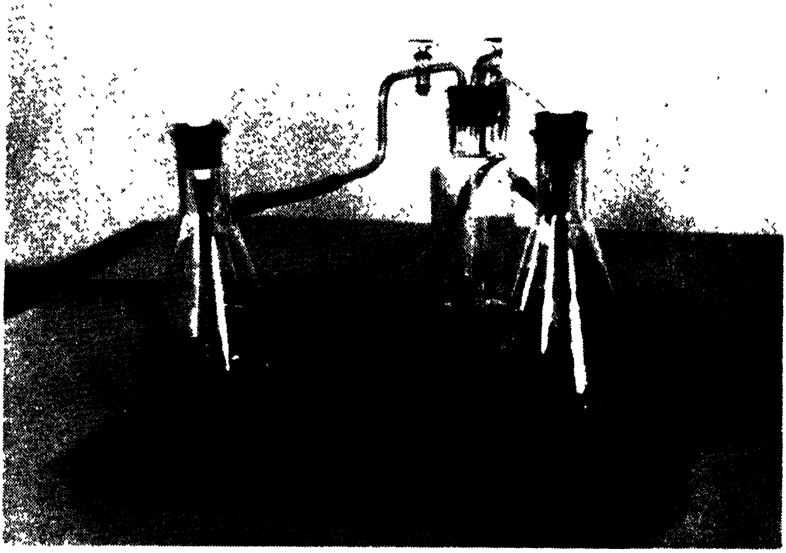

FIG. 2.—A convenient filtering arrangement.

(b) Other portions of each tissue were extracted for 10 hours in Soxhlet extractors with such volumes of 95% ethanol that the water present in the samples diluted the concentration of ethanol in the final solutions to about 80% by volume.

5. *Filtering the extracts.*—All filtrations were made with Büchner funnels using suction. The filtering arrangement shown in Figure 2, also devised in the authors' laboratory (3), was used throughout the work. With the aqueous extracts transfers and washings were made with distilled water, and with the alcoholic extracts 80% ethanol was used for these purposes. With some tissues much difficulty was encountered in filtration especially with the aqueous extracts. Therefore, "filter-aid" was used[1] to facilitate this operation. Although the use of this material aided filtration to some extent, in many cases it was necessary to use disks cut from filter cloth instead of filter paper to make filtration possible. The alcoholic extracts from the Soxhlet extraction required no filtration.

6. *Evaluating the amounts of sugar in the extracts.*—The alcohol was removed from the alcoholic extracts by evaporation on the steam bath. The protein was precipitated from all extracts with lead acetate, and excess lead was removed with potassium oxalate except with aliquots to be titrated with ceric sulphate, in which cases disodium orthophosphate was used for this purpose. Separate aliquots were hydrolyzed with hydrochloric acid and invertase, respectively. The invertase solution used was obtained from Difco Laboratories, Inc., Detroit, Mich. The amounts of total sugar present in aliquots of the extracts from each sample and each treatment were evaluated by four different procedures: *viz.*, (a) The gravimetric method and (b) the volumetric thiosulfate method, respectively, of the Association of Official Agricultural Chemists (4); (c) the cuprous titrimetric method of Shaffer and Hartmann (5); and (d) the ceric sulphate titrimetric method of Hassid (6).

DISCUSSION

Certain manipulative operations in the analytical procedures used warrant a brief discussion. Use of the Waring Blendor for extracting sugars from plant tissue as described possessed advantages in economy of time and labor compared to the use of Soxhlet extractors or hand mortars, respectively, for this purpose. Trouble encountered in filtering the extracts of certain plant tissues, however, appeared at first to be a serious disadvantage of the practice, but the use of 80% ethanol instead of water as the extracting solvent for some tissues, the substitution of filter cloth for filter paper, and the addition of filter-aid helped to mitigate this difficulty.

It was found to be most advantageous to mix the filter-aid with the extract immediately before filtration was begun. The amount required varied from 1 to 5 grams, depending upon the kind of tissue being analyzed. Experiments showed that the filter-aid used by the authors did not adsorb perceptible amounts of sugar.

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[1] Johns Manville Hyflo Super-Cel.

In the gravimetric procedure Selas filtering crucibles of No. 10 porosity were used in most cases for removing the cuprous oxide from the final solution. Such crucibles were convenient and ordinarily very satisfactory for this purpose. The rate of filtration was usually rapid enough to permit the solutions to be filtered within the time schedule required by this procedure; however, with extracts of onion bulbs in which invertase was used the final solutions were extremely difficult to filter. In these cases it was necessary to replace the Selas crucibles with Gooch crucibles containing very thin pads of asbestos.

In using the ceric sulphate titration method for evaluating sugars as published by Hassid (6) the tissue extracts were clarified with finely divided carbon before they were titrated. Hassid recommended that the clarification process be carried out before hydrolysis; however, the authors noted in preliminary work that if the tissue extracts were clarified prior to hydrolysis, values for total sugar were markedly lower than those obtained if the extracts were clarified after the hydrolytic treatment. Hence, the latter practice was followed in obtaining all data reported in this paper.

The results in Table 1 show that the values for sugars obtained by the four evaluation procedures are generally in good agreement when aliquots of a given extract were subjected to the same hydrolytic treatment. Moreover, it will be noted with the first three crops that for a given method of extraction, and a given method of hydrolysis, duplication of values for sugars in different weighed portions of tissue is excellent for each evaluation procedure. Also, judging from the results with the first three crops, it appears that the solvents and methods of extraction used were equally effective in removing sugars from the tissues and that hydrolysis with hydrochloric acid was as efficient as with the invertase preparation used.

Young brome grass, however, is an exception to these general tendencies, since the percentages of total sugar apparently present in this tissue appears to have been greatly influenced by the solvent and hydrolytic agent used. It will be noted than when water was used as the solvent and hydrochloric acid as the hydrolytic agent the values for sugar are consistently high. The next highest values were obtained with aqueous extracts and invertase, and the lowest values resulted when invertase was used with extracts from the Soxhlet extractors. The same tendencies are evident in the results from spinach leaves, but to a less striking extent.

Apparently these variations in results are due to the fact that brome grass and spinach leaves contained substances which were more soluble in water than in 80% ethanol and yielded reducing sugars more readily when hydrolyzed with acid than with invertase. According to Archbold (7) and Hassid (8) certain water-soluble polysaccharides behave in this manner.

The variable results obtained with young brome grass and spinach

TABLE 1.—*Results*

Per cent of total sugar in crop plants as determined by different methods of extraction and evaluation[1]

KIND OF CROP	METHOD OF EXTRACTION[2]	METHODS USED TO EVALUATE SUGAR							
		A.O.A.C.				SHAFFER-HARTMANN		CERIC SULPHATE TITRATION	
		GRAVIMETRIC		VOLUMETRIC					
		A[3]	In[4]	A	In	A	In	A	In
Carrot roots	I	7.70	7.71	7.68	7.67	7.29	7.52	7.95	7.79
	I	7.63	7.56	7.63	7.50	7.33	7.44	7.75	7.78
	II	7.39	7.39	7.34	7.27	7.08	7.18	7.46	6.90
	II	7.39	7.43	7.32	7.35	7.04	7.20	7.12	6.96
	III	7.29	7.42	7.19	7.02	7.26	7.20	7.90	7.64
	III	7.39	—	7.37	7.34	7.33	7.33	8.13	7.69
Onion bulbs	I	4.80	4.93	4.51	4.53	4.88	4.89	4.61	4.45
	I	4.87	5.06	4.50	4.55	4.92	4.94	4.71	4.45
	II	4.75	4.87	4.52	4.38	4.83	4.86	4.64	4.34
	II	4.61	4.74	4.36	4.49	4.83	4.80	4.87	4.28
	III	4.57	4.35	4.45	4.31	4.60	4.42	4.31	4.40
	III	4.74	4.50	4.57	4.42	4.78	4.48	4.43	4.50
Tomato fruits	I	2.36	2.26	2.39	2.34	2.37	2.34	2.41	2.20
	I	2.36	2.47	2.33	2.36	2.39	2.34	2.35	2.17
	II	2.44	2.44	2.34	2.41	2.46	2.44	2.29	2.28
	II	2.30	2.32	2.40	2.39	2.44	2.45	2.35	2.28
	III	2.38	2.31	2.34	2.34	2.31	2.26	2.23	2.26
	III	2.35	2.47	2.31	2.24	2.20	2.14	2.25	2.23
Spinach leaves	I	.58	.64	.51	.48	.50	.49	.45	.44
	II	.51	.48	.48	.45	.49	.48	.44	.45
	III	.45	.47	.44	.44	.47	.46	.47	.44
Young brome grass	I	2.21	1.66	2.17	1.82	2.20	1.77	2.20	1.70
	II	1.72	1.36	1.67	1.47	1.68	1.48	1.53	1.35
	III	1.39	1.22	1.34	1.13	1.47	1.29	1.42	1.24

[1] The values in each horizontal line are from separate aliquots of the same extract of one weighed portion of sample. In the vertical columns each value represents a different weighed portion of each tissue. Values given for spinach and young brome grass are averages of results from duplicate aliquots of the same extract.

[2] I = Waring Blendor, distilled H_2O as solvent; II = Waring Blendor, 80% ethanol as solvent; and III = Soxhlet extractor, ethanol as solvent

[3] Hydrolysed with HCl. Five ml conc. acid to 100 ml aliquot, heated in boiling water bath exactly 10 min. (Results same as with 15–20 hours at room temp.)

[4] Hydrolysed with invertase.

leaves typify the complexities likely to be encountered in the determination of sugars in crop plants and emphasize the need for sufficient preliminary work with each different crop to insure that the values obtained with a given analytical procedure actually represent the sugar content of the tissue.

Only values for total sugar are given in this paper, but it is obvious that reducing sugars present in the tissue extracts could have been evaluated in aliquots not subjected to hydrolysis.

CONCLUSIONS

(1) A Waring Blendor equipped with improvised blending vessels was convenient and effective for extracting sugars from the crop plants analyzed.

(2) Water and 80% ethanol could be used interchangeably for extracting sugars from some crop plants, but for others extraction with water gave higher results.

(3) Under the same conditions of extraction and hydrolysis, the four methods used for evaluating reducing sugars gave approximately the same results for total sugars in each crop plant analyzed.

(4) The use of carbon for clarification of extracts prior to hydrolysis was found to give lower results for total sugars than when the carbon was used subsequent to such hydrolysis.

(5) The complexities encountered in the determination of sugars in crop plants necessitate sufficient preliminary work with each different crop to insure that the value obtained with a given analytical procedure represents the true sugar content of the tissue.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the efficient assistance given them in this work by Mrs. Elva D. Jones of the Department of Agricultural Chemistry and by Dr. Gloria D. Manalo, former associate in the Department of Agricultural Chemistry and at present a member of the faculty of the University of the Philippines, Manila, P. I.

LITERATURE CITED

(1) *This Journal.* **25,** 573 (1942).
(2) *Ind. Eng. Chem., Anal. Ed.,* **16,** 717 (1944).
(3) *Chemist-Analyst,* **32,** 40 (1943).
(4) *Methods of Analysis, A.O.A.C.,* 6th Ed. (1945), pp. 572–573.
(5) *J. Biol. Chem.,* **45,** 365 (1920).
(6) *Ind. Eng. Chem., Anal. Ed.,* **8,** 138 (1936).
(7) *Ann. Botany, N. S.,* **2,** 183 (1938).
(8) *J. Am. Chem. Soc.,* **61,** 1223 (1939).

THE DETERMINATION OF THE SULFIDE-SULFUR CONTENT OF CALCIUM SILICATE SLAGS IN RELATION TO THEIR NEUTRALIZATION VALUE*

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A knowledge of the sulfur content of agricultural slags is of importance both because of its value as a plant nutrient and its effect upon the chemical evaluation of the neutralization capacity of the slags. Where the neutralization value of a slag is computed from the analyses of Ca and Mg, such value is subject to a minus correction for the *total sulfur* content of that slag. If, however, the neutralization capacity of the slag is obtained through *titrative procedures*, this value is subject to a minus correction for the *sulfide sulfur* content only.

The sulfide correction problem was dealt with briefly in our early contribution on titrative procedures for calcium silicate slags (6, p. 332) and subsequently in a report on liming materials (7, pp. 296 and 306). Schollenberger (3) had suggested that "automatic" correction for sulfide sulfur be provided through oxidation by means of hydrogen peroxide in the dissolvent acid. This proposed modification was considered of doubtful value towards improving the accuracy and as an undesirable complication for a routine procedure. The titration to *p*H 5.2 using Bromcresol green, therefore, was recommended for adoption for the determination of the neutralization value of blast furnace slags, with the annotation: "Without correction for sulfide content" (2, p. 306). It was suggested, also, that a deduction of 3.5 per cent calcium carbonate-equivalence from the titration value might be admissible as a reasonable mean value for sulfide content. In some instances, however, it may be desirable to obtain a more exact correction which would be based on the sulfide content of the slag.

The objectives of the present investigation were to acquire information relative to the sulfide and sulfate content of various representative agricultural slags and to evolve a simple and accurate procedure for the determination of their sulfide content.

EXPERIMENTS ON THE JOINT DETERMINATION OF SULFIDE CONTENT AND NEUTRALIZATION VALUE

The most convenient procedure for the determination of the sulfide content would seem to be the collection of hydrogen sulfide that is evolved in the process of dissolution of the slag in the determination of its neutralization value. The apparatus required to carry out this process was:

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* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 11–13, 1948.

a 60-ml separatory funnel fitted into a 250 Erlenmeyer flask; a 25×150 mm test tube provided with inlet and outlet tubing connected to flask by means of 6-inch length of 3/16-inch rubber tubing. In the preliminary dissolution of the slag at room temperature, the reaction flask was shaken either intermittently by hand or continuously by mechanical device and the absorbent tube was attached to the flask by means of a rubber band, which was removed when the flask was placed over a Bunsen burner, so that the absorbent tube could be extended for immersion in cold water in a 600-ml beaker.

Dissolution in Presence of Air

Charges of one-half gram of slag were placed in 250-ml Erlenmeyer flasks; the sides of flasks were washed down with about 5 ml of water, the stopper that carried the separatory funnel and the outlet tube was fitted tightly into the reaction flask. A charge of 25 ml of a 2 per cent cadmium sulfate solution was placed in the absorbent tube, which was connected to flask as described. Standard acid, 35 ml, was introduced into the flask through the separatory funnel while the suspension was being swirled. Further agitation was by hand at one minute intervals, or continuously on a shaking machine for 10 to 15 minutes. The funnel was rinsed with 10 ml of water, which was drawn into flask. The contents then were boiled 10 minutes by means of a small Bunsen burner. The rubber connections then were snapped off from the cadmium sulfate tube quickly to prevent the backing-up of the cadmium sulfide suspension.

Dissolution of Charge in Atmosphere of Carbon Dioxide

Since sulfide oxidation by air in the dissolution train was deemed a possible source of error, a parallel series of slag dissolutions were made in air, and in an atmosphere of carbon dioxide, in the same manner and with the same apparatus as described above. The carbon dioxide was introduced after the slag charge had been wetted, and was led through the train for 5 minutes at the rate of 500 ml per minute prior to dissolution of the slag by the acid.

Sulfide Sulfur Evolved from Slags through Dissolution in 0.5 N Hydrochloric Acid

The separatory funnel was rinsed, the neutralization value of the slag was determined by the titration procedure of the flask to *p*H 5.2 using Bromcresol green (7), and the evolved sulfide value was measured by the acidity engendered in cadmium sulfate absorbent tubes.

The eleven samples of representative blast furnace slags analyzed for sulfide content were furnished by the Standard Slag Company of Youngstown, Ohio. They had been used in the testing of the simplified titration procedure to *p*H 5.2 against Bromcresol green, as reported in 1946 (7), "without correction for sulfide content." The results obtained by dissolution in 0.5 N acid are given in Table 1. The calcium carbonate-equivalence of the sulfide content ranges from 1.7 to 4.2 per cent and gives a mean value of 2.50 per cent. The sulfide values are reproducible within 0.2 ml titration, or 0.2 per cent calcium carbonate-equivalence, which is satisfactory in the evaluation of the neutralization capacity of slags. The few slight increases shown as a result of dissolution being effected in an atmosphere of carbon dioxide were not sufficient to warrant the extra effort.

TABLE 1.—*Sulfide sulfur evolved from blast furnace slags through dissolution in 0.5 N HCl, and determined through titration of the $CdSO_4$ absorbent*

SLAG*	TITRATIONS, ML OF 0.1 N NaOH PER 0.5-G CHARGE									
	BY DISSOLUTION IN ATMOSPHERE OF CO_2					BY DISSOLUTION IN AIR				
	1	2	3	AV.	S	1	2	3	AV.	S
					per cent					*per cent*
A	3.80	3.80	3.80	3.80	1.22	3.80			3.80	1.22
B	4.25	4.30		4.28	1.37	4.15			4.15	1.33
C	2.50	2.50	2.50	2.50	0.80	2.50			2.50	0.80
D	3.15	3.10		3.13	1.00	3.05			3.05	0.98
E	1.90	1.90		1.90	0.61	1.80			1.80	0.58
F	2.10	2.05		2.08	0.67	2.20	2.00	2.00	2.07	0.66
G	2.40	2.35	2.40	2.39	0.76	2.30	2.25	2.30	2.27	0.73
H	2.15	2.10		2.13	0.68	2.15	2.05	2.05	2.07	0.66
I	2.00	2.00		2.00	0.64	2.05	2.00	2.00	2.02	0.65
J	1.80	1.70	1.80	1.77	0.57	1.70	1.65	1.65	1.67	0.53
K	2.40	2.30	2.30	2.33	0.75	2.35	1.95	2.00	2.10	0.67
Average				2.57	0.82				2.50	0.80

* Supplied by Standard Slag Co., 1945 shipment.

Completeness of Sulfide Evolutions and Accuracy of the Sulfide Determination

The accuracy of the sulfide procedure was tested by the use of a prepared cadmium sulfide, and by checking the sum of the sulfide value indicated by the evolutions and residual sulfur against the directly determined total sulfur content of eleven slags. The prepared sulfide was obtained by passage of hydrogen sulfide through a water scrubber and into a 2 per cent solution of cadium sulfate. The precipitate was filtered and washed with water until the washings were neutral to methyl orange.

Experiments with Cadmium Sulfide

To effect complete dissolution of the prepared cadmium sulfide for determination of its sulfide value, it was necessary that the hydrochloric acid be more concentrated than 0.5 *N*. A (1+4) concentration of the dissolvent hydrochloric acid effected dissolution of the prepared sulfide upon heating. The volatility of hydrochloric acid at this concentration necessitated the use of a scrubber between the evolution flask and the cadmium sulfate. A 25×150 mm test tube, half-filled with water and maintained at near boiling, was utilized for that purpose. With these two variations, the apparatus and procedure used for the adsorption of hydrogen sulfide in cadmium sulfide were those described. Usually the dissolution was effected in the presence of the contained air, but in some experiments a hydrogen atmosphere was produced by the inclusion of 1 gram of zinc dust with the cadmium sulfide charge. The cadmium sulfide was analyzed for total sulfide and residual sulfur after sulfide evolution.

TABLE 2.—*Analysis of the CdS prepared and used as absorbent for H_2S*

CdS CHARGE	SULFIDE SULFUR			SULFATE SULFUR		SUM OF $S^{=}+SO_4^{=}$ SULFUR	TOTAL S DETERMINED
	0.1 *N* TITRATION	S-EQUIVALENCE					
		PER DETERMINATION	IN SAMPLE	PER DETERMINATION	IN SAMPLE		
g	*ml*	*g*	*per cent*	*g*	*per cent*	*per cent*	*per cent*
.100	12.40	.01984	19.84				
.100	12.30	.01968	19.68				
.100	12.30	.01968	19.68				
Average			19.73				
.050	6.15	.00984	19.68	.00045[b]	0.90	20.58	
.050	6.20	.00992	19.84	.00045	0.90	20.74	
.050	6.25	.01000	20.00	.00044	0.88	20.88	
Average			19.84		0.89	20.73	
.025	3.10	.00496	19.84				
.025	3.10	.00496	19.84				
.025	3.10	.00496	19.84				
Average			19.84				
.100[a]	12.50	.02000	20.00	.00085[c]	0.85	20.85	20.94[d]
.100	12.60	.02016	20.16	.00085	0.85	21.01	20.82
.100	12.55	.02008	20.08	.00087	0.87	20.95	21.00
Average			20.08		0.86	20.94	20.92

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[a] Sulfide evolution of this group was in an atmosphere of hydrogen, all other evaluations were in the contained air.

The corresponding $BaSO_4$ weights were for—
[b] .0033, .0033, and .0032 g
[c] .0062, .0062, and .0063 g } all corrected for a .0008-g reagent blank.
[d] .1532, .1523, and .1536 g

Results from the Use of Prepared Cadmium Sulfide

The triplicate titration values given in Table 2 show good reproducibility for specific cadmium sulfide charges, and precision for charges in the range between .025 and .100 g and those charges embraced the sulfide contents of 1-g charges of blast furnace slag. Slightly higher results were obtained from the .100-g charges of cadmium sulfide, plus zinc dust. This may mean that some oxidation of the sulfide to sulfur did occur in the limited air volume. The highest sulfide value obtained on the prepared product was, however, only 20.08 per cent, which is 2.12 per cent less than the theoretical value. The sulfate residue from the charge of cadmium sulfide accounted for only 0.9 per cent of the sulfur content of the cadmium sulfide. The sum of the sulfide and sulfate forms of sulfur was 20.94 per cent and is in accord with the 20.92 per cent total content found by the direct determination. The findings reported in Table 2 show the accuracy and precision of the titration procedure for sulfide sulfur, especially when the hydrogen sulfide evolution was accompanied by the evolution of hydrogen.

TABLE 3.—*Effect of nascent hydrogen upon the recovery of sulfide sulfur from blast furnace slabs by the evolution method*

SLAGS*	A—BY 0.5 N/HCl IN AIR			B—BY HCl, 1+4, IN HYDROGEN†			
	0.1N TITRATION	$CaCO_3 \Bumpeq$ OF S	SULFIDE S	0.1 N TITRATION	$CaCO_3 \Bumpeq$ OF S	SULFIDE S	$CaCO_3 \Bumpeq$ B−A
	ml	*per cent*	*per cent*	*ml*	*per cent*	*per cent*	*per cent*
A	3.80	3.80	1.22	7.90	3.95	1.26	.15
B	4.15	4.15	1.33	8.80	4.40	1.41	.25
C	2.50	2.50	0.80	5.40	2.70	0.86	.20
D	3.05	3.05	0.98	6.40	3.20	1.02	.15
E	1.80	1.80	0.58	4.85	2.43	0.78	.63
F	2.07	2.07	0.66	6.10	3.05	0.98	.98
G	2.27	2.27	0.73	5.70	2.85	0.91	.58
H	2.07	2.07	0.66	4.80	2.40	0.77	.33
I	2.02	2.02	0.65	5.35	2.68	0.86	.66
J	1.67	1.67	0.53	4.85	2.43	0.78	.76
K	2.10	2.10	0.67	7.30	3.65	1.17	1.55
Average		2.50	0.80		3.07	0.98	0.57

* Supplied by Standard Slag Company, Youngstown, Ohio, 1945 shipment.
† By reaction with zinc dust.

Although the prepared cadmium sulfide was precipitated under analytical conditions, the precipitate was found to contain cadmium sulfate and approximately 10 per cent of sorbed non-sulfide matter. This does not support the suggestion (5, p. 912) that the gravimetric determination of the filtered precipitate can be used as a check upon the titration results of the sulfide determination.

Accuracy of the Procedure for the Evolution of Blast Furnace Slags

Because of the apparent incompleteness in the evolution of sulfide from cadmium sulfide in ordinary atmosphere, zinc dust was included in sulfide determinations on the eleven slags, which circumstance precluded simultaneous determinations of the neutralization value and sulfide content on the same charge. Charges of 1 gram per 50 ml of hydrochloric acid (1+4) were used when zinc was included. Apparatus and procedure were as described for the cadmium sulfide, except that preliminary contact in the cold with frequent agitation was adhered to. The sulfide values given in Table 3 were obtained through dissolution with (1+4) hydrochloric acid and nascent hydrogen, in comparisons with dissolution in 0.5 *N* hydrochloric acid under the conditions prescribed for the determination of neutralization value.

Effect of Nascent Hydrogen upon Sulfide Recovery

The results given in Table 3 show that the use of nascent hydrogen gave an average increase in sulfide values of .57 per cent calcium carbonate-equivalence, although some of the increases were as much as 1.55 per cent. Since the use of zinc also introduced a greater acid concentration, the effect of the nascent hydrogen should be differentiated from the effect of acid concentration. Triplicate sulfide determinations on slag K were made by dissolution in hydrochloric acid (1+4) without addition of zinc. The obtained values of 2.2 per cent calcium carbonate-equivalence are in close agreement with those effected by dissolution of the slag in 0.5 *N* hydrochloric acid. Hence, the increase of from 2.10 to 3.65 per cent calcium carbonate-equivalence in slag K is ascribed as an effect of the nascent hydrogen rather than an effect of acid concentration. Apparently, however, the lesser values are not due to oxidation by the contained air, since carbon dioxide displacement failed to effect any material increase in the sulfide values. The chemical composition and structure of the slag are probably the factors that govern sulfide recovery. It is noteworthy that the four charges that showed the least effect from analytical addition of zinc were the water-quenched or air-granulated slags, A, B, C, and D, of "glassy" structure and ready dissolvability by hydrochloric acid in either of the concentrations used. In contrast, the air-cooled slags underwent dissolution slowly and invariably yielded more hydrogen sulfide when the charges were fortified by additions of zinc. Apparently the zinc served to effect reduction of the higher oxides of manganese, and possibly those of iron, which might induce oxidation of the generated hydrogen sulfide and thereby preclude atmospheric oxidation in the apparatus.

Hence, to assure the complete evolution of the sulfide sulfur from an hydrochloric acid-dissolved charge of blast furnace slag, it is deemed imperative to fortify the charge with zinc, or with a metal that will assure dissolution with an adequate supply of nascent hydrogen.

PROCEDURE FOR THE DETERMINATION OF THE SULFIDE SULFUR CONTENT OF CALCIUM SILICATE SLAGS

REAGENTS

(a) *HCl* (1+4).

(b) *Zinc dust.*—C. P. grade, of low lead content.

(c) *Absorbent.*—Dissolve 20 g of $CdSO_4 \cdot 2\frac{2}{3}\ H_2O$ in H_2O and make to 1 liter. Adjust reaction to *p*H 5.6 by means of either potentiometric titration or colorimetric comparisons on a separate 50-ml aliquot and matching a buffer of same *p*H.

(d) *Standard alkali.*—Prepare 0.1 *N* NaOH from CO_2-free NaOH solution and use of CO_2-free H_2O.

(e) *Standard acid.*—0.1 *N* HCl.

APPARATUS

A 250-ml Erlenmeyer evolution flask to fit No. 5.5 stopper, a 60-ml separatory funnel fitted into the stopper and the lower third of its stem drawn to a 2 mm opening and bent upwards at its extremity. The funnel in the stopper is adjusted so that its stem is ¼ inch from the bottom of the evolution flask. Two 25 × 150 mm test tubes are fitted with No. 4 two-hole stoppers and inlet and outlet of 6 mm tubing, which serve to contain jointly the 50 ml of absorbent. The tubes are in series and should be placed in a 600-ml beaker filled with cold water, and the beaker placed upon a tripod on level with the evolution flask. Another test tube of similar size is half-filled with H_2O and clamped in position between evolution flask and absorbent solution to provide trap for HCl vapor.

DETERMINATION

Weigh 1 g of minus 80-mesh slag into the evolution flask; add 1 g of Zn dust and wash down sides with 5–10 ml H_2O; mix contents by means of flattened end of stirring rod and connect the flask. Introduce 50 ml of HCl (1+4) into separatory funnel; open stopcock to allow acid to flow into reaction flask, swirling the contents meanwhile. If necessary, apply pressure to effect complete transfer of acid from funnel. Close stopcock and continue the swirling 5 min. at brief intervals. Apply heat; continue the swirling until boiling starts and then regulate flame to maintain active boiling, although not too vigorous, throughout 10 min. Keep the contents of the HCl vapor-trap near the boiling point throughout dissolution of the slag, then snap off the rubber tubing at the $CdSO_4$ intake quickly, taking care not to allow a backing-up of the contents. Cut off heat, discard contents of the evolution flask and of the scrubber tube, and set up apparatus for the next determination.

Filter the CdS suspension on a 9 cm gravity filter leading into a 250 ml Erlenmeyer flask and wash with H_2O to an overall volume of 100 ml. Add 4 drops of a 0.2 per cent soln of methyl red and agitate vigorously while titrating slowly to the exact orange-yellow tint of the reference soln. That soln comprises 50 ml of the CdS soln diluted to 100 ml, with identical concentration of indicator and contained in a 250-ml Erlenmeyer flask. Should the end point be passed with resultant precipitation of $Cd(OH)_2$, introduce 1–2 ml of 0.1 *N* HCl, and allow to stand until the precipitate disappears and then complete the titration dropwise under vigorous agitation. Divide by 2 the ml of net 0.1 *N* NaOH used to obtain the per cent $CaCO_3$-equivalence of the sulfide sulfur in the sample. The ml of 0.1*N* NaOH × .0016 = g sulfide sulfur per determination; and the latter, times 100 = percentage of sulfide S.

TABLE 4.—*Summations of separately determined occurrences of sulfide sulfur and sulfate sulfur, in comparison with total sulfur determinations on blast furnace slags*

SLAG[a]	TOTAL SULFUR				FRACTIONAL DETERMINATIONS OF SULFUR				SULFUR DISPARITY[d]	$CaCO_3 \Leftrightarrow$ OF NON-SULFIDE S CONTENT
	AS $BaSO_4$ FROM 1 GM.					SULFATE S				
	1	2	AV.	S	SULFIDE[b] S	AS $BaSO_4$ FROM 1 GM.	S[c]	SUM		
	g	*g*	*g*	*per cent*	*per cent*	*g*	*per cent*	*per cent*	*per cent*	*per cent*
A	.1010	.1029	.1020	1.40	1.26	.0031	.03	1.29	.11	.44
B	.1115	.1132	.1124	1.54	1.41	.0043	.04	1.45	.09	.40
C	.0680	.0690	.0685	0.94	0.86	.0038	.04	0.90	.04	.25
D	.0815	.0815	.0815	1.12	1.02	.0027	.02	1.04	.08	.31
E	.0790	.0800	.0795	1.09	0.78	.0197	.26	1.04	.05	.97
F	.1241	.1268	.1255	1.72	0.98	.0497	.67	1.65	.07	2.31
G	.0871	.0887	.0880	1.21	0.91	.0162	.21	1.12	.09	.94
H	.0744	.0753	.0749	1.03	0.77	.0156	.20	0.97	.06	.81
I	.0824	.0826	.0825	1.13	0.86	.0136	.17	1.03	.10	.84
J	.1270	.1291	.1281	1.76	0.78	.0696	.94	1.72	.04	3.06
K	.1273	.1311	.1292	1.77	1.17	.0389	.52	1.69	.08	1.88
Average				1.34	0.98		.28	1.26	.07	
Extremes:										
Low				.94	.77		.02			
High				1.77	1.41		.94			

[a] Furnished by Standard Slag Company, 1945 shipment.
[b] Data taken from Table 3, Section B.
[c] S was computed from $BaSO_4$ weights after correction for reagent blank of .0011 g $BaSO_4$.
[d] Sulfur unaccounted for when "summations" were subtracted from determinations of totals.

NON-SULFIDE SULFUR IN SLAGS

Distinction has been drawn between total sulfur content and that in the form of sulfide, in relation to the two methods of analysis that are used for determination of the neutralization value of slags. The slags used in the sulfide methods were used in an effort to establish the actual quantitative differences between the two kinds of sulfur combinations that occur in various slags. Their total sulfur content was determined, and the fractions that remained in solution or in suspension after evolution of sulfide sulfur were determined also, so that the aggregates of the separate determinations would provide overall checks against totals. The total sulfur results given in Table 4 were obtained through aqua regia digestions of 1-g charges, dehydration and removal of silicon dioxide, reduction of ferric iron with alunimum powder, and precipitation as barium sulfate. Residual sulfur was determined through dehydration of residues from the experiments of Table 3, Section B, redissolving and brominating, and then proceeding as for total sulphur.

The sulfide sulfur values for the water-quenched slags are given in Table 4 and are virtually identical with those found as totals. This is in contrast to the disparity between the total and sulfide sulfur values found for the air-cooled slags. The differences range from 1 to 3 per cent of calcium carbonate-equivalence, and are accounted for almost entirely by the sulfates found in the residues. In general, difference between the values for total sulfur and those obtained as summations of the sulfide and residual forms was $0.07 \pm .03$ per cent sulfur. This small difference may be due to incomplete oxidation by the bromination imposed during the evaporation of the sulfide residuals.

ADDITIONAL SLAG ANALYSIS FOR SULFIDE AND SULFATE SULFUR

Samples of blast furnace slags used in previous studies were available for additional sulfide analyses and these were supplemented by 6 electric-furnace phosphate reduction slags. The results assembled in Table 5 substantiate the earlier finding that the sulfur in the granulated blast furnace slags is nearly all in the sulfide form, whereas sulfates account for substantial fractions of the sulfur contained by the air-cooled slags. The sulfur content of the electric furnace phosphate-reduction slags is only about one-tenth that of the sulfur content of the blast furnace slags. The mean sulfide sulfur content of 26 blast furnace slags was found to be 3.35 per cent, in terms of calcium carbonate-equivalence.

EFFECT OF SULFUR CORRECTION UPON THE CHEMICAL EVALUATION OF THE NEUTRALIZATION POWER OF SLAGS

As noted, there are two types of procedure for the chemical evaluation of the liming effectiveness of slags. In the direct analysis, the calcium and magnesium content is reckoned as of basic character and is balanced against the sum of the equivalents of sulfur, fluorine, and phosphorus, to establish the potential neutralization capacity of the material. This type of analysis may be taken as standard. The other procedure is an adaptation of the simple alkalimetric method with a prescribed titration end-point to minimize the sorbtion of bases by the precipitates of gelatinous silica and hydrated alumina. In a contribution on slag titrations (6, p. 326) it was pointed out that because of the variant silicon dioxide/aluminum oxide ratio in slags, *such titrations do not attain the accuracy of the standard method of analysis closer than within 1 to 2 per cent.*

TABLE 5.—*Sulfur as sulfide and sulfate and their $CaCO_3$-equivalences in blast furnace slags and in slags from electric rock phosphate reduction furnaces*

SLAG			SULFUR AS SULFIDE		SULFUR AS SULFIDE		$CaCO_3$≎ OF SUMMATIONS
SOURCE	NO.	PROCESSING	S	$CaCO_3$≎	S	$CaCO_3$≎	
			per cent	*per cent*	*per cent*	*per cent*	*per cent*
Blast furnace[a]	1	Granulated	1.68	3.25	.05	0.15	5.40
	2	"	1.79	5.60	.06	0.18	5.78
	3	"	1.19	3.70	.05	0.15	3.85
	4	Air Cooled	1.15	3.60	.44	1.38	4.98
	5	"	0.98	3.05	.25	0.78	3.83
	6	"	1.09	3.40	.45	1.41	4.81
	7	"	1.18	3.70	.25	0.78	4.48
	8	"	0.66	2.05	.25	0.78	2.85
	9	"	1.26	3.95	.37	1.16	5.11
	10	"	0.93	2.90	.09	0.28	3.18
	A	"	0.82	2.55	N.D.	—	—
	B	"	0.98	3.05	"	—	—
	C	Granulated	0.94	2.95	"	—	—
	D	"	1.19	3.73	"	—	—
Reserve Sample		"	0.88	2.75	"	—	—
Electric furnace[b]	1053	Quenched	0.15	0.48	"	—	—
	1117	"		0.30	.02	.06	.36
	1224	"	0.13	0.40	N.D.	—	—
	1259	"	0.11	0.35	"	—	—
	1286	"	0.19	0.60	"	—	—
	1291	"	0.18	0.55	"	—	—
Average for blast furnace slags				3.48			
Average for 26 blast furnace slags, including those in Table 3, B				3.35			

[a] Supplied by Standard Slag Company, 1942 samples from 1 to 10; 1945 from A to D.
[b] From Tennessee Valley Authority operations at Wilson Dam, Albama.

Since the official method (1) prescribed for limestone was found inapplicable to slags, the simplified procedure of titration to *p*H 5.2 using Bromcresol green was proposed as tentative method for the evaluation of blast furnace slags (7, p. 297). Schollenberger (4) has proposed that the alkalimetric procedure be modified to include special provisions for the oxidation of the sulfur present as sulfides in slag. His procedure requires a more complicated apparatus and a more involved manipulative technique. However, it lays claims to desired improvements, the principal ones being the elimination of interference effects from the silica-alumina precipitate at the titration point of *p*H 8.0, and the automatic correction for sulfide by oxidation by means of hydrogen peroxide prior to the titration.

The analyses presented in Table 6 were for eleven slags by means of six procedures used in previous studies, with corrections for either sulfide or total sulfur content. The criterion of accuracy is the concordance between values given in each column with those reported in Column II. The Bromcresol green ("B.C.G.") results of Column IV show an average deviation of 0.9 per cent, 7 of the values being within 1 per cent and 4 being within 2 per cent. In this laboratory equally good results were obtained through the use of Schollenberger's 2-stage titration method *without sulfide oxidation.* The results in Column VI were obtained by Schollenberger through the use of his own procedure, *with sulfide oxidation.* Those findings register no improvement in accuracy, and show deviations greater than those of any of the other methods under study. Moreover, five of the eleven analyses by Schollenberger show calcium carbonate-equivalence differences of from 2.6 per cent to 4.6 per cent between duplicates, whereas, in the use of the direct titration to *p*H 5.2, such differences were within 0.5 per cent. Hence, the results by the proposed procedure, with inclusion of the step of sulfide oxidation, fall short of the precision obtainable by the simple titrative procedure to *p*H 5.2 by "B.C.G.," where an allowance of a mean value of 3.5 per cent calcium carbonate-equivalence for sulfide sulfur has been made. The titrations to *p*H 5.2 by "B.C.G.," as in Column VII of Table 6, show a mean deviation of only 1.0 per cent. In ten of the eleven findings the deviation is between 1 and 2 per cent, which is within the tolerance inherent to a titrative procedure on a sample from large bulk, as was indicated in a related contribution (6).

PRACTICAL CONSIDERATION AS TO CHOICE OF CHEMICAL PROCEDURE FOR SLAGS

For a product to be classified as a liming material, it is reasonable that a minimal neutralization value be prescribed. However, it is not likely that the salability of a classified liming material would be affected materially by a 2 per cent variation in the determination of neutralization value, so long as the indicated value is well beyond the required minimum.

TABLE 6.—*Neutralization values for blast furnace slags, in terms of $CaCO_3$, as determined by various procedures, with corrections for sulfide sulfur content in the titrative procedures*

SLAG[a]	DETERMINED $CaCO_3$≎ Ca+Mg MINUS S			TITRIMETRIC DETERMINATIONS									
				III INDIRECT		DIRECT				VI AT pH 8.2, WITH "AUTOMATIC" CORRECTION		VII AT pH 5.2, AFTER A −3.5%[a] CORRECTION FOR MEAN SULFIDE≎	
	I PRODUCER'S		II OURS			IV AT pH 5.2		V AT pH 8.2					
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
A	89.2	+1.1*	88.1	88.3	+0.2*	88.9	+0.8*	89.4	+1.3*	87.5	−0.6*	89.3	+1.2*
B	83.7	+0.3	83.4	83.3	−0.1	83.2	−0.2	82.8	−0.6	83.1	−0.3	84.1	+0.7
C	86.6	+0.3	86.3	86.7	+0.4	87.4	+1.1	87.3	+1.0	87.8	+1.5	86.6	+0.3
D	79.9	+0.2	79.7	79.9	+0.2	80.3	+0.6	80.0	+0.3	80.6	+0.9	80.0	+0.3
E	93.6	−0.7	94.3	95.0	+0.7	94.5	+0.2	94.4	+0.1	94.1	−0.2	93.4	−1.1
F	86.9	+1.1	85.8	86.0	+0.2	87.7	+1.9	87.1	+1.3	83.7	−2.1	87.2	+1.4
G	85.0	+0.9	84.1	84.4	+0.3	86.1	+2.0	85.5	+1.7	84.6	+0.5	85.4	+1.3
H	80.0	−0.6	80.6	80.5	−0.1	80.5	−0.1	80.7	+0.1	81.2	+0.6	79.4	−1.2
I	77.1	(+3.7)	73.4	73.3	−0.1	74.3	+0.9	74.2	+0.8	75.1	+1.7	73.5	+0.1
J	81.5	+0.2	81.3	79.4	−1.9	80.9	−0.4	80.7	−0.6	81.5	+0.2	79.8	−1.5
K	84.1	+0.8	83.3	83.7	+0.4	84.5	+1.2	83.6	+0.3	85.7	+2.4	84.7	+1.4
Average deviation, regardless of sign		0.6			0.4		0.9		0.7		1.0		1.0
Deviation Limits						Numbe r of Deviations							
0 −0.5		4			9		4		4		4		3
0.6–1.0		4			1		3		4		3		1
1.1–2.0		2			1		4		3		2		7
2.1–3.0											2		

[a] Supplied by Standard Slag Company, 1945 samples.
I. Analysis of Ca+Mg by Standard Slag Company laboratories, minus our sulfide determination.
II. Our Ca+Mg analysis after dissolution in 0.5 *N* HCl.
III. Joint titration of Ca+Mg, after elimination of SiO_2 and Al_2O_3 (6, p. 330).
IV. According to procedure, *This Journal*, 30, p. 297, Method II, with bromcresol green.
V. Our analyses according to Schollenberger's 2-stage titration procedure, but without addition of H_2O_2, *This Journal*, 30, p. 298, Method III, with phenolphthalein.
VI. Analyses by Schollenberger according to his latest procedure, with his automatic correction obtained through the oxidation of sulfide before the titration, *This Journal*, 30, 117–124.

* The deviations were from comparisons between the results obtained by each method with those given in Column II.

In case the titration of a liming material should register a value considerably below that minimum, the liming material would be classified as substandard. This would serve to segregate the few slags that might register border-line values within 1 to 2 per cent of the standard requirement, and therefore require a more exact analytical technique to assure correct value and proper classification. Exactness within 2 per cent of the true value of the slag is not attained by the Schollenberger titration procedure or any other titrative method yet tested. That value is obtained only upon careful determination of the contents of calcium, magnesium, and sulfur.

SUMMARY

The evolution and determination of the sulfide sulfur content of slags were investigated to ascertain whether correction should be applied to the titrations used to measure the neutralization value of slags.

Cadmium sulfide was prepared and used to establish precision and accuracy of the titration of the acidity engendered in a cadmium sulfate absorbent.

Upon additions of hydrochloric acid of either 0.5 *N*, or (1+4) concentration, evolution of the sulfide from slags was incomplete in presence of air or in an atmosphere of carbon dioxide. Complete evolution was obtained from the cadmium sulfide and from the slags, however, when the slag plus zinc dust was treated with (1+4) hydrochloric acid. The findings prompted the proposal of a simple method for the evolution and titration of the sulfide sulfur content of calcium silicate slags.

The several proposed procedures for the determination of the neutralization value of blast furnace slags were compared upon basis of precision and accuracy, with special attention given to the merits of the sulfide oxidation as a step in the titrative procedure. The automatic sulfide correction failed to produce the expected improvement in the accuracy possessed by the direct titration procedure to *p*H 5.2, and in the precision attainable by the direct procedure.

It is concluded that the simple titration procedure to *p*H 5.2 by Bromcresol green should be adhered to as an equitable and practical means for the determination of the proximate neutralization capacity of a blast furnace slag.

A correction of 3.5 per cent calcium carbonate-equivalence is suggested as a reasonable deduction for sulfides. A procedure for sulfide determination in slags is presented, however, for occasions where more exact correction for sulfide is required.

REFERENCES

(1) *Methods of Analysis, A.O.A.C.*, 6th Ed. (1945), p. 42 [3.4].

(2) Jacob, K. D., Correspondence, August 1945.

(3) Schollenberger, C. J., Correspondence in spring of 1945.

(4) ———. "Titration Method for the Potential Soil Neutralizing Power of Blast Furnace Slags," *This Journal*, **30**, 117 (1947).
(5) Scott, W. W., "Standard Methods of Chemical Analysis," 5th Ed. (1939). Van Nostrand Company, New York.
(6) Shaw, W. M., "Titrative Determination of the Neutralization Value of Calcium Silicate Slags," *This Journal*, **28**, 310 (1945).
(7) ———, Report on Liming Materials. *This Journal*, **30**, 295 (1947).

MILK FAT IN MILK CHOCOLATE*

By L. W. Ferris (Food and Drug Administration, Federal Security Agency, Buffalo, N. Y.)

The problem of the accurate determination of the amount of milk fat in milk chocolate has been before the Association of Official Agricultural Chemists since the early days of enforcement of the Food and Drug Act. At that time two procedures for removing the fat from milk chocolate were under consideration. One was to shake the sample with ether, or petroleum ether, until all fat that could be dissolved was extracted; and the other was to extract the sample for 18 hours in a continuous extracting apparatus with anhydrous ether, interrupting the process at some stage to grind the sample, thus making the fat more accessible to the ether. In commenting on these methods Associate Referee W. L. Dubois (1) in his report to the Association in 1910 noted that lower results were obtained by a straight ether extraction and assumed that merely shaking the sample with ether did not dissolve all the milk fat, since he had found results on a milk powder alone extremely low. He reported analyses of four samples of milk chocolate, the milk fat being estimated from the Reichert Meissl number of the extracted fat. Total milk solids calculated were compared with the amount stated by the manufacturer to be present, but no report was made of the per cent of milk fat actually present.

In 1926, collaborative work (2) was done on one sample of milk chocolate of known composition. The fat for the determination of Reichert Meissl number was obtained by shaking the sample with two portions of petroleum ether. Results of the collaborators show a variation equal to about 36 per cent of the amount of milk fat present.

In 1935, Hillig (3) presented a "unified method" using hydrochloric acid and petroleum ether for the determination of fat in foods. Two determinations were necessary to obtain sufficient fat for one determination of Reichert Meissl number.

In 1945, M. Offutt (4) reported total fat on one sample of milk chocolate by the Hillig method and by a "modified Roese-Gottlieb method," using hydrochloric acid, alcohol, and a mixture of petroleum and ethyl ethers. Offutt recommended that further work be done on these two methods.

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W. O. Winkler (5) has suggested two procedures for the complete removal of fat from milk chocolate. One makes use of alcohol and petroleum ether, and the other of ammonia, alcohol, ether, and petroleum ether with massive quantities of solid ammonium chloride and magnesium nitrate to prevent unbreakable emulsions. No analytical results were reported.

It is generally agreed that the Reichert Meissl number, being uniformly high on milk fat and low on other fats, offers the best available criterion for estimating the amount of milk fat in a mixed fat. Efforts in the past have been directed to devising a procedure that would remove all the fat from milk chocolate in as pure a state as possible and, at the same time, yield at least 5 grams of fat: the amount necessary for a determination of the Reichert Meissl number. The procedure of the Association of Official Agricultural Chemists for the separation from milk chocolate of fat to be used for the Reichert Meissl number has always called for a straight ether extraction. The only changes made from the first edition to the present sixth edition have been in the amount of sample to be used, and the substitution of ethyl ether for petroleum ether.

The purpose of this paper is to present evidence that a straight ether extraction on milk chocolate may leave unextracted an appreciable amount of milk fat, and to offer a method for the complete removal of fat from milk chocolate. A single determination of total fat yields a quantity sufficient for duplicate determinations of the Reichert Meissl number.

METHOD

Place 40 g of milk chocolate in a centrifuge bottle, add 100 ml of ether and shake vigorously until the chocolate is well broken up. Centrifuge, and decant or syphon off the ether layer. Make two more extractions with 100 ml of ether each, combine the extracts, and evaporate most of the ether. Add to the residue in the centrifuge bottle 25 ml of 1.4 dioxane and heat for 20 min. in a water bath at approximately 90°C, with frequent stirring. Cool, add 100 ml of ether, stopper, shake well, centrifuge, and decant. Make two more extractions with 50 ml ether, combine the extracts, evaporate the ether, and remove the dioxane completely with a current of air on the steam bath. Dissolve the fat in ether, filter into the flask containing the preliminary ether extract, evaporate ether, dry at 100° and weigh. If any insoluble material appears in the dry fat, filter in a hot oven before weighing out the 5-g sample for the Reichert Meissl number.

RESULTS

In a preliminary experiment, 99 per cent of the total fat present in a sample of milk powder was removed from 6 grams by the dioxane ether treatment. By making a double dioxane and ether treatment, the same completeness of fat extraction was secured on an 18-gram sample, giving sufficient fat for the Reichert Meissl determination. The Reichert Meissl number found was 28.4.

That the fat remaining after a straight ether extraction of chocolate may be mostly milk fat, is shown by the following results on a commercial sample of "ivory coating," made with whole milk powder, cocoa butter, and flavor:

TABLE 1.—*Results*

METHOD	FAT PERCENTAGE	REICHERT MEISSL NUMBER OF FAT
Straight ether extraction	39.1	5.0
Dioxane-ether on residue	1.0	26.1

The Reichert Meissl number on a sample of chocolate liquor containing no milk fat was 0.1 by the straight ether extraction of fat, and 0.3 on that obtained by the proposed method.

Table 2 gives results on two commercial milk chocolates, five milk chocolate coatings and one "ivory milk coating." The table permits a comparison between the A.O.A.C. method and the proposed method, both as to total fat and milk fat calculated from the Reichert Meissl number.

TABLE 2.—*Comparison of 2 methods (per cent)*

SAMPLE NO.	PRODUCT	MILK FAT*	TOTAL FAT		MILK FAT†		INCREASE BY PROP. METHOD	
			A O.A.C. METHOD	PROPOSED METHOD	A.O.A.C. METHOD	PROPOSED METHOD	TOTAL FAT	MILK FAT
1	Milk Chocolate	—	32.6	33.9	4.9	6.0	1.3	1.1
2	" "	—	33.4	33.6	5.3	5.4	0.2	0.1
3	Milk Chocolate Coating	—	33.4	33.5	3.3	3.4	.2	.1
4	" " "	—	32.1	32.4	2.8	3.1	.3	.3
5	" " "	—	30.5	30.8	3.2	3.6	.3	.4
6	" " "	3.8	37.2	37.6	3.9	4.1	.4	.2
7	" " "	4.0	32.7	33.2	3.9	4.2	.5	.3
8	"Ivory Milk" Coating	7.2	38.9	39.8	6.5	7.2	.9	.7

* Calculated from manufacturer's formula.
† Calculated from Reichert Meissl number of fat by formula (6).

CONCLUSION

It is shown that a straight ether extraction of milk chocolate as in the A.O.A.C. method leaves unextracted a small amount of fat, which is principally milk fat. A method is presented which is rapid, easy in manipulation, and effects a more complete extraction of the fat. In one instance slightly over one per cent more of milk fat was obtained by the proposed method.

REFERENCES

(1) Bureau of Chemistry Bul. 152, pp. 159–163 (1912).
(2) *This Journal*, **9**, 462 (1926).

(3) *Ibid.*, 18, 454 (1935).
(4) *Ibid.*, 28, 482 (1945).
(5) Unpublished report, Food and Drug Administration, Federal Security Agency, Washington, D. C.
(6) *Methods of Analysis*, 6th Ed. (1945), sec. 19.30.

DETERMINATION OF WATER INSOLUBLE FATTY ACIDS IN DRIED EGGS*

By FRED HILLIG (Food Division,† Food and Drug Administration, Federal Security Agency, Washington 25, D. C.)

The development of acids in eggs has been recognized as accompanying certain types of decomposition. Dried eggs prepared from liquid eggs which had become sour before drying were found to contain volatile and lactic acids.[1] Acidity of the ether extract has also been applied to dried eggs, and directions for its determination have been included in the methods of the A.O.A.C.[2] The acids determined by the latter method include all of the non-volatile fatty acids, as well as the major part of any lactic acid that may be present. However, the method does not measure those acids which are present in eggs as an ether-insoluble compound, such as inorganic salts. A method for the determination of water insoluble fatty acids (hereinafter referred to as WIA) has already been proposed for cream and butter.[3] This method has now been applied to dried eggs. The procedure follows:

DETERMINATION OF WIA IN DRIED EGGS

Weigh 2 g of the prepared sample into a 100 ml beaker and make into a uniform paste with a small quantity of water, using a heavy stirring rod. Transfer the material to a 250 ml centrifuge bottle with water, using a total of 25 ml for the entire operation, and shake vigorously. Rinse the beaker with 20 ml of alcohol, transfer the rinsings to the centrifuge bottle, shake vigorously, and add 50 ml of ether.

Proceed as directed in the method for cream and butter.[3]

RECOVERY EXPERIMENTS

The quantities of oleic and palmitic acids, in alcoholic solutions, shown in Table 1, were transferred to centrifuge bottles containing 2 g of authentic, sound, dried eggs, reconstituted with water, and the WIA were determined by the above method. Table 1 shows the very satisfactory recoveries obtained.

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* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 11-13, 1948.

† W. B. White, Chief.

[1] Lepper, H. A., Bartram, M. T., and Hillig, F., "Detection of Decomposition in Liquid, Frozen, and Dried Eggs," *This Journal*, 27, 204 (1944).

[2] *Methods of Analysis, A.O.A.C.*, 6th Ed. (1945). Sec. 23.29–23.32.

[3] Hillig, F., "Determination of Water-Insoluble Fatty Acids in Cream and Butter," *This Journal*, 30, 575 (1947).

TABLE 1.—*Recovery of oleic and palmitic acids added to 2 g of dried eggs*

SAMPLE NO.	WIA ADDED			WIA FOUND		RECOVERED
	OLEIC	PALMITIC	TOTAL	TOTAL	NET*	
	mg	*mg*	*mg*	*mg*	*mg*	*per cent*
1	282	256	538	533.8	525.4	97.7
				540.4	532.0	98.9
2	141	256	397	388.0	379.6	95.6
				397.4	389.0	98.0
3	282	128	410	403.2	394.8	96.3
				396.4	388.0	94.6
4	70.5	64.0	134.5	142.1	133.7	99.4
				142.8	134.4	99.9
5	70.5	32.0	102.5	112.2	103.8	101.3
				112.6	104.2	101.7
6	35.3	64.0	99.3	108.0	99.6	100.3
				109.1	100.7	101.4
7	28.2	25.6	53.8	59.9	51.5	95.7
				58.1	50.7	94.2
8	14.1	25.6	39.7	47.9	38.5	96.9
				47.7	39.3	99.0
9	28.2	12.8	41.0	48.2	39.8	97.1
				49.9	41.5	101.2
10	56.4	25.6	82.0	91.5	83.1	101.3
				91.5	83.1	101.3

* The blank on each sample was 8.4 mg (average of 4 analyses).

The method was also applied to a number of the dried egg samples which were the subject of the previous study.[1]

The data given in Table 2 show that dried edible eggs contain small quantities of WIA. No material increase in WIA was observed when edible liquid eggs were held for one day under refrigeration, or for short periods at high temperature before drying; or when they were frozen, and then thawed before drying. Overheating in drying did not appear to increase the WIA. There was also no decided increase when lower grade current receipt eggs were held for 18 hours at 85°F.; but, when addled eggs and mixed rots were added to different batches of the same type of eggs and held for from 18 to 23 hours, there was a decided increase in the WIA, one sample inoculated with mixed rots having an acid content of almost 11 per cent. That limited amounts of certain types of decomposed eggs may

TABLE 2.—*WIA in authentic dried*† *eggs*

SAMPLE NO.	GROUP 1—EDIBLE EGGS	ODOR LIQUID EGGS	WIA
			mg/100 g
A-1	Better grade current receipts	none	415
A-2	Better grade current receipts	none	420
A-3	Better grade current receipts held 22 hours at 35°F.	none	380
B-1	Lower grade current receipts	none	540
B-2	Lower grade current receipts	none	915
B-3	Lower grade current receipts held 20 hours at 35°F.	none	530
B-4	Lower grade current receipts held 6 hours at 85°F.	"off"	443
RF-1	Current receipts frozen and thawed for 24 hrs. at 70°F.	none	193
RH-2	Current receipts frozen and thawed for 48 hrs. at 70°F.	none	478
RH	Current receipts overheated by drying at 510°F.	none	230
	GROUP 2—EGGS HELD AT 85°F.		
B-5	Lower grade current receipts held 18 hours	sour	728
RO-2	Lower grade current receipts with 2.5% added odorless addled eggs held 22 hours	sour	2,270
RO-3	Lower grade current receipts with 5.0% added odorless addled eggs held 18 hours	sour	4,483
RR-2	Lower grade current receipts with 2.5% mixed rots held 23 hours	sour	5,065
RR-3	Lower grade current receipts inoculated with mixed rots	sour	10,780
	GROUP 3—CONTAINING ADDED INEDIBLE EGGS		
RO-1	Current receipts with 5% odorless addled eggs	none	2,420
RR-1	Current receipts with 5% mixed rots	none	950
RN	Current receipts with 10% moldy	moldy	855
RB	Current receipts with 0.3% black rots	putrid	298
RU	Current receipts with 2% musty	musty	343
BL	Weak yolk and blood ring eggs	none	540

† The description of the preparation of these samples has previously been given.[1]

be present in dried eggs without being detected by the method, is shown by the data obtained on Group 3 eggs.

WIA IN COMMERCIAL DRIED EGGS

Data obtained on eggs dried on a commercial scale and known to be of good quality are given in Table 3.

The WIA content is within the range found for the authentic edible eggs in Table 1.

SUMMARY

An adaptation of the method for the determination of water-insoluble fatty acids in cream and butter is proposed for the determination of these

TABLE 3.—*WIA in commercial dried eggs of edible quality*

SAMPLE NO.	WIA	SAMPLE NO.	WIA
	mg/100 g		*mg/100 g*
1	325	12	205
2	350	13	280
3	550	14	290
4	540	15	120
5	485	16	185
6	225	17	120
7	265	18	155
8	255	19	330
9	230	20	195
10	205	21	55
11	590		

acids in dried eggs. It has been shown that small amounts of WIA are found in authentic dried edible eggs, but that when liquid eggs are allowed to become sour before drying, the WIA in the dried product are increased several fold. Commercial dried eggs of acceptable quality were found to contain WIA in the range found for authentic dried edible eggs.

SUCCINIC ACID IN DECOMPOSED EGG PRODUCTS

By HENRY A. LEPPER and FRED HILLIG (Division of Food,* Food
and Drug Administration, Federal Security
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During the course of the studies by Claborn and Patterson[1] which resulted in the methods for the determination and identification of lactic and succinic acids, it was observed that dried eggs, prepared from raw material known to be decomposed, contained significant amounts of succinic acid. It is known that succinic acid results from certain types of microbial decomposition in foods,[2] coming either from carbohydrate breakdown, or from the decomposition of aspartic and glutamic acids, themselves the products of protein hydrolysis. It thus seemed in order to test this new and very specific method for succinic acid as an index of the various types of decomposition known to occur in eggs, before or after breaking out. In all of the experiments described below, the Claborn and Patterson method for succinic acid was followed.

Shell eggs of edible, marketable quality were purchased on three different occasions. A dozen eggs were broken out, well mixed, and immediately analyzed for succinic acid. In no case was any detected. These samples are identified as 1, 2, and 3, respectively in Table 1. Sample No. 3 was so chosen that each egg had a substantial amount of dirt from the nest adhering to its shell.

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* W. B. White, Chief.

[1] *This Journal*, 31, 134 (1948).

[2] Porter, J. R., *Bact. Chem. & Physiol.*, 1946, pp. 810–813, 815, 862 and 864.

The liquid egg mix from sample No. 3 was permitted to stand at room temperature ((approximately 85°F.). When observed 22 hours later, it was found to be in a state of active decomposition, being covered with foam and having a pronounced sour odor. The decomposition must have started some time before. Analysis of a sample from the mix showed that substantial amounts of succinic acid had developed at this stage. The remainder of the mix was permitted to stand 24 hours longer at room temperature at which time the foaming and sourness had definitely increased. This advancing decomposition was reflected in an increased succinic acid

TABLE 1.—*Succinic acid in liquid and dried eggs*

SAMPLE NO.	DESCRIPTION	SUCCINIC ACID
		mg per 100 g[*]
1	Shell eggs, edible marketable quality	none
2	Shell eggs, edible marketable quality	none
3	Shell eggs, edible marketable quality (dirty shells)	none
4	Sample 3 held 22 hours at 85°F.—sour	9.8[1]
5	Sample 3 held 46 hours at 85°F.—sour	19.0[1]
6	Shell eggs—mixed rots	3.3[1]
7	Shell eggs—white rots	13.2[1]
8	Shell eggs—black rots	24.4[1]
9	Shell eggs—addled, no odor	none
A-1	Dried eggs from better grade current receipts	none
A-2	Dried eggs from better grade current receipts	none
B-4	Dried eggs from lower grade current receipts, held 6 hours at 85°F.—not sour	none
B-5	Dried eggs from lower grade current receipts, held 18 hours at 85°F.—sour	56[2]
RO-2	Dried eggs from lower grade current receipts, plus 2.5% addled odorless eggs, held 22 hours—sour	38[2]
RO-3	Dried eggs from lower grade current receipts, plus 5% addled odorless eggs, held 18 hours—sour	44[2]
RR-2	Dried eggs from lower grade current receipts, plus 2.5% mixed rots, held 23 hours—sour	130[2]
RR-3	Dried eggs from lower grade current receipts, inoculated with mixed rots—sour	118[3]

[*] The analytical results reported in this table were obtained by H. V. Claborn, formerly of the Food and Drug Administration.

[1] The identity of succinic acid was confirmed by the crystalline form of its barium salt.

[2] The isolated succinic acid was identified by conversion to its p-toluidide.

[3] The isolated succinic acid had the same m p. as authentic acid.

TABLE 2.—*Market shell eggs*

SAMPLE NO.	PURCHASED IN	GRADE	NO. BAD	SHELL COLOR*	LOCALITY OF PRODUCTION	REMARKS
75-166-7 H	San Francisco, Calif.	AA	0	W	Petaluma, Calif.	
75-176 H	San Francisco, Calif.	AA	0	W	Petaluma, Calif.	
75-175 H	San Francisco, Calif.	A	16	W	Petaluma, Calif.	7 days old when collected; 14 days in transit.
72-104 H	Los Angeles, Calif.	AA	0	W		Also examd. frozen.
72-180 H	Los Angeles, Calif.	A	9	W		7 days old & 7 days in transit.
78-661 H	Seattle, Wash.	AA	0	B	Woodinville, Wash.	Day-old farm stock.
78-662 H	Seattle, Wash.	AA	0	W	N. W. Wash.	Store stock
86-365 H	Denver, Colo.	AA	0	W	nearby Denver	
86-367 H	Denver, Colo.	A	0	W	15 mi. west Denver	
86-368 H	Denver, Colo.	A	0	W	40 mi. east Denver	
65-297 H	Philadelphia, Pa.	A	0	W	nearby Pa. eggs	
65-298 H	Philadelphia, Pa.	B	3	W & B	Minnesota eggs	10 days old—also examd. frozen.
65-299 H	Philadelphia, Pa.	C	4	W & B	Minnesota eggs	10 days old.
89-664 H	Baltimore, Md.	A	0	W	Harford Co., Md.	Also examd. frozen.
85-374 H	Baltimore, Md.	B	0	W	Harford Co., Md.	
85-375 H	Baltimore, Md.	C	3	W	Harford Co., Md.	Also examd. frozen.
92-866 H	Boston, Mass.	A	0	B	Mass. eggs	Also examd. frozen.
92-802 H	Boston, Mass.	B	0	B	Conn. eggs	Also examd. frozen.
92-803 H	Boston, Mass.	C	0	B	Maine, N. H., & Vt. eggs	
7-815 K	Buffalo, N. Y.	A	0	W	Erie Co., N. Y.	Also examd. frozen—not over 8 days old.
7-816 K	Buffalo, N. Y.	B	0	W	Erie Co., N. Y.	Also examd. frozen.
6-701 K	Buffalo, N. Y.	C	10	W	Erie Co., N. Y.	
9-935 K	New York, N. Y.	A	0	W	South Jersey	
9-936 K	New York, N. Y.	B	1	W	Callicoon Center, N. Y.	
9-938 K	New York, N. Y.	C	1	W	Sullivan Co., N. Y.	
55-498 H	Atlanta, Ga.	A	0	W	Cumming, Ga.	
54-899 H	Atlanta, Ga.	B	1	W	Jackson, Ga.	Also examd. frozen.
54-900 H	Atlanta, Ga.	B	11	W	Ellijay, Ga.	

TABLE 2.—*Continued*

SAMPLE NO.	PURCHASED IN	GRADE	NO. BAD	SHELL COLOR*	LOCALITY OF PRODUCTION	REMARKS
23-215 K	New Orleans, La.	A	1	B	Kansas	
23-068 K	New Orleans, La.	A	2	W	Kansas	
23-072 K	New Orleans, La.	B	7	W	Kansas	
27-816 K	St. Louis, Mo.	A	0	W	St. Louis Co., Mo.	
27-817 K	St. Louis, Mo.	B	0	W	New Haven, Mo.	
27-818 K	St. Louis, Mo.	B	2	W	St. Marys, Mo.	
69-046 H	Chicago, Ill.	A	0	W	Forrest, Ill.	Also examd. frozen.
69-043 H	Chicago, Ill.	B	0	B	Forrest, Ill.	
69-044 H	Chicago, Ill.	C	0	B	Forrest, Ill.	
24-752 K	Minneapolis, Minn.	A	0	W	Forest Lake, Minn.	
24-920 K	Minneapolis, Minn.	B	8	W	Northfield, Minn.	
24-737 K	Minneapolis, Minn.	B	1	W	Cedar Mills, Minn.	
21-380 K	Kansas City, Mo.	A	0	W	within 150 mi.—K. C.	
21-381 K	Kansas City, Mo.	B	4	W	within 150 mi.—K. C.	
21-382 K	Kansas City, Mo.	B	2	W	within 150 mi.—K. C.	
84-283 H	Cincinnati, Ohio	A	0	W	west central Ohio	
84-284 H	Cincinnati, Ohio	B	1	W	west central Ohio	
84-285 H	Cincinnati, Ohio	C	2	W	west central Ohio	

* W = white; B = brown.

content. The results of the two samples of mix are given as Nos. 4 and 5, respectively, in Table 1.

To study the occurrence of succinic acid in eggs which become decomposed in the shell, a supply of rejects were secured from the candling operations of a dealer in shell eggs. The individual eggs were broken and classified into four groups as to condition. The first three groups cover the types of decomposed eggs usually encountered, namely mixed rots, white rots, and black rots. Each of these types contained succinic acid (samples No. 6, 7, and 8, respectively). The fourth group contained eggs which on breaking showed a ruptured yolk more or less addled into the white. However, they had no odor. Such eggs are usually high in bacteria and will develop an offensive odor of decomposition if allowed to remain in the shell. No succinic acid was detected in this group (sample No. 9).

The application of the method to dried eggs was studied on a series of authentic samples manufactured from liquid eggs of known condition. The results are included in Table 1. Two samples, representing a good commercial grade of shell eggs, promptly dried after breaking out, showed no determinable succinic acid (samples No. A-1 and A-2). Another sample (No. B-4), manufactured from the lowest grade of current receipt eggs, which were broken and held six hours at 85°F. before drying, also showed no succinic acid. There was no decomposition evident in this sample, either in the liquid or in the dried state. However, dried egg sample No. B-5, made from the same liquid magma as B-4 after it was allowed to stand an additional 12 hours at 85°F before drying, until obviously sour and unfit for food, showed large amounts of the acid. Sample Nos. RO-2, RO-3, RR-2, and RR-3 were prepared by inoculating liquid eggs from four separate lots of lower grade current receipts of shell eggs as indicated in the tabulation, and drying the magma after it had been held long enough to become sour.

To demonstrate further the absence of detectable amounts of succinic acid in edible marketable shell eggs, samples were purchased on the retail market in sixteen different cities. Food and Drug Administration inspectors in each city were requested to purchase three samples of 2 dozen eggs each, representing respectively the best, the medium, and the lowest grade of eggs available on the local market. Thirty-seven samples consisted of white shell, and seven of brown shell varieties; two samples were white and brown (one dozen each). The eggs were packed to obviate breakage and sent, properly sealed and identified, together with the records of collection, to Washington for analysis. Upon receipt, the eggs from each sample were broken and the contents individually noted and smelled. Eggs of inedible condition or quality were rejected. The edible eggs in each case were then composited, thoroughly mixed, and analyzed for succinic acid. No succinic acid was found in any of the 46 samples.

Without exception the samples containing a substantial proportion of inedible eggs were either of the lower grades or were known to be a week old, with an additional exposure to summer temperature because of delay in transit. It is a fair assumption that the eggs from such samples that were classified as edible were on the verge of becoming inedible, since they were of comparable age and had been exposed to the same conditions as the inedible eggs in the sample. It is thus significant that even such eggs gave no evidence of succinic acid development.

With a number of samples the egg magma, after withdrawal of the sample for analysis, was immediately frozen and held at 0°F. for from two to three months. Ten such samples of frozen eggs were thawed and analyzed, and in no case was succinic acid detected. The grades indicated on the carton in which the eggs were purchased, or on the inspector's collection report, were found to be as stated. The details of the samples discussed above are summarized in Table 2. The grades assigned are those established by the U. S. Department of Agriculture.[3]

SUMMARY AND CONCLUSIONS

Succinic acid was not found in shell eggs of acceptable, edible quality or in frozen or dried eggs prepared from such shell eggs. It is formed during some processes of decomposition of eggs, either in the shell or after separation from the shell. This evidence of decomposition is demonstrable in dried eggs.

Appreciation is extended to M. T. Bartram of the Food and Drug Administration for assistance in identifying and classifying the eggs according to the type of decomposition, and to W. I. Patterson for helpful suggestions during the course of these studies. The cooperation of the Food and Drug Inspectors in the collection of authentic samples is also appreciated.

WATER INSOLUBLE FATTY ACIDS IN CREAM AND BUTTER*

By FRED HILLIG[1] and S. W. AHLMANN[2] (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.)

INTRODUCTION

There are three principal sources of supply of cream for butter making: (1) whole milk delivered direct to the creamery by the farmer, where it is separated and churned, (2) cream separated on the farm where, in some

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[3] U S. Standards for Quality of Individual Shell Eggs, Federal Register, 13, 1359 (March 16, 1948).

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TABLE 1.—*Acids in cream and butter*

SAMPLE IDENTIFICATION	INCUBATION PERIOD	CREAM			BUTTER				
		ML 0.1 *N* ACID PER 100 G			ML 0.1 *N* ACID PER 100 G				
		ACETIC	BUTYRIC	LACTIC	TOTAL	ACETIC	BUTYRIC	LACTIC	ACIDITY OTHER THAN LACTIC AND VOLATILE
	Days								
A	0	0.1	None	Trace	1.8	0.6	None	None	1.3
	14	26.9	11	153	35.1	4.8	None	29.0	1.3
B	0	0.5	None	None	3.4	None	None	None	3.4
	7	28.5	50	132	38.0	5.0	5.1	14.7	13.2
Sweet Cream Butter					5.0	0.1	None	None	4.9
Commercial, #1					19.0	2.3	0.3	6.9	9.5
Commercial, #2					52.0	3.2	2.1	17.3	29.4

cases, it may be held for several days, or even weeks, unrefrigerated, before it is delivered to the cream gathering station to be held until it is collected by the centralizer, and (3) farm separated cream which may be held at the farm until a sufficient quantity is accumulated for direct delivery to the creamery or centralizer.

Lactic acid producing bacteria are always present in such cream, and the quantity and rate at which lactic acid will be formed depends upon the sanitary conditions under which the cream is produced and upon the time and the temperature at which it is held. Normal lactic acid souring has long been recognized by some sections of the industry as desirable in the manufacture of butter, in fact souring is often induced under controlled conditions through the addition of "starters" containing lactic acid producing bacteria. However, it frequently happens with improperly controlled spontaneous souring that other changes occur which render the cream unsuitable for use in the preparation of a food product for human consumption. This latter type of cream, and the butter made therefrom, presents a problem to the regulatory official. This paper is a report of progress on an investigation of the chemical changes taking place in cream constituents during initial lactic acid souring, and in cream in which additional deteriorating changes have occurred.

EXPERIMENTAL

Raw sweet cream was incubated at 90°F., with daily stirring. A portion of the cream was churned at the beginning and another at the end of the incubation period. Volatile and lactic acids were determined on both the cream and butter samples by methods previously described (1, 2). The butter was also analyzed for total acids as follows: 50 grams of butter were weighed into each of two 250-ml centrifuge bottles, 10 ml of water were added and the mixture was gently warmed to melt the butter. The contents of the bottles were neutralized with *N* sodium hydroxide, using phenolphthalein as indicator. The fat was extracted with 50 ml each of ether and petroleum ether. The residues in the centrifuge bottles were made acid with sulfuric acid and sodium tungstate was added. They were then transferred separately to a lactic acid extractor (2) and extracted for three hours. The extracts from the two 50-g portions of butter were combined, about 50 ml of water were added and the material was titrated with 0.1 *N* sodium hydroxide. This gave the total acidity on 100 grams of butter.

Data on 2 samples of cream and butter churned therefrom and on an authentic sweet cream butter and two samples of butter obtained on the market, are given in Table 1.

The sum of the titers of the volatile and lactic acids subtracted from the titer of the total acids gives the acidity due to unidentified acids. In the case of butter A, there was no increase in the acidity due to these

TABLE 2—*Authentic butter—1944*

SAMPLE NO.	VAT ACIDITY AS LACTIC	CLASSES OF CREAM IN CHURN				WIA ON FAT BASIS	MOLD
		0	1	2	3		
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*mg/100 g*	*per cent*
1	0.14	100				94	0
2	0.32	92	8			100	0
3	0.85	79	21			184	47
4	0.45	71	29			85	33
5	0.77	71	29			100	15
6	0.72	57	43			174	8
7	0.76	53	47			184	45
8	0.56	49	51			88	61
9	0.62	48	52			114	28
10	0.63	42	58			86	32
11	0.76	41	59			101	58
12	0.76	38	62			133	10
13	0.85	34	66			84	20
14	0.77	30	70			100	3
15	0.80	28	72			169	22
16	0.70	21	79			99	11
17	0.75	20	80			88	5
18	0.55	74	24	2		185	10
19	0.56	53	45	2		100	2
20	0.90	44	54	2		399	66
21	0.95	35	63	2		599	59
22	0.83	20	78	2		531	22
23	0.75	15	83	2		374	46
24	0.84	32	65	3		189	68
25	1.05	25	72	3		273	42
26	0.74	25	72	3		523	43
27	1.04	14	82	4		443	47
28	0.95	13	83	4		379	50
29	0.40	70	25	5		75	17
30	0.84	12	82	6		293	84
31	0.93	25	68	7		359	58
32	0.97	10	82	8		191	22
33	0.71	19	71	10		353	37
34	0.80	9	80	11		428	77
35	1.27	29	53	18		269	53
36	0.63	9	73	18		350	97
37	0.66		90	10		133	7
38	1.00	47	49	3	1	380	43
39	—	10	74	14	2	259	91
40	0.95		74	24	2	338	86
41	1.00	8	77	12	3	248	63
42	0.87		71	26	3	546	34
43	1.05		59	37	4	300	83

unidentified acids over an incubation period of 14 days, although large quantities of lactic and acetic acids were formed in the cream, indicating extensive souring. However, butter B showed a marked increase of unidentified acids. The cream from which it was churned at the seventh day of incubation showed lactic and butyric acids in relatively large quantities, butyric acid being the principal volatile acid produced.

These results indicate a relationship between the occurrence of these unidentified acids in butter and the condition of the cream from which it was churned. A study of this acidic material, using the method developed for isolating and determining water insoluble acids (3) (hereafter referred to as "WIA"), showed it to consist chiefly of these acids. The method was then applied to commercial creams and butters.

TABLE 3.—*Authentic butter—1945*

SAMPLE NO.	CLASSES OF CREAM IN CHURN				WIA ON FAT BASIS	MOLD
	0	1	2	3		
	per cent	*per cent*	*per cent*	*per cent*	*mg/100 g*	*per cent*
1	100				50	18
2	82	18			145	74
3	66	34			140	64
4	61	39			154	40
5	34	66			104	44
6	76	23	1		61	20
7		96	4		163	76
8	2	88	10		315	64
9	5	73	22		374	82
10		71	27	2	543	82
11		61	30	9	565	96

Cream is judged as to condition principally by taste and odor both by the industry and by State and Federal regulatory agencies. In this investigation the classification of creams proposed by Vandaveer and Wildman (4) was followed in establishing the condition of the creams churned into the various butters studied. The description of the classifications used is as follows:

(0) Cream which is either sweet or good clean sour; sound cream which a discriminating housewife would use for butter making in her own kitchen.

(1) Cream which has an indefinite or indefinable "off" flavor or odor (*e.g.*, due to feeds).

(2) Cream in which a decomposition characteristic, ("cheesiness," rancidity, putridity, etc.), is strong enough to be definitely recognizable.

(3) Cream in which the flavor characteristics of decomposition are markedly stronger than creams classified as (2).

TABLE 4.—*Authentic butter—1946*

SAMPLE NO.	VAT ACIDITY AS LACTIC	CLASSES OF CREAM IN CHURN				WIA ON FAT BASIS	MOLD
		0	1	2	3		
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*mg/100 g*	*per cent*
1	—	100				56	7
2	0.12	100				76	0
3	—	100				78	4
4	0.17	100				93	0
5	0.15	100				104	0
6	0.32	100				115	4
7	—	100				119	0
8	—	100				135	2
9	0.38	55	45			159	2
10	0.50		100			100	1
11	0.45		100			193	2
12	0.68		98	2		133	21
13	0.81	11	87	2		209	51
14	0.90	4	94	2		200	37
15	0.82	6	92	2		261	53
16	0.87	4	93	3		150	10
17	0.95	13	84	3		273	86
18	0.85		97	3		209	79
19	0.80	2	94	4		203	68
20	0.57		95	5		128	21
21	1.14		94	6		315	76
22	0.85	10	84	6		331	79
23	0.94		93	7		394	76
24	0.45	2	88	10		201	2
25	0.80		89	11		269	17
26	0.81		84	16		371	5
27	0.92		80	20		231	81
28	0.77		76	24		248	21
29	1.15		90	9	1	411	93
30	1.00		83	16	1	350	24
31	0.58		90	8	2	260	15
32	1.50		66	32	2	381	2
33	0.85		72	26	2	426	55
34	1.22		73	25	2	448	92
35	1.38		65	30	5	410	100
36	1.05		67	19	14	425	95

During the summer of 1944, 43 authentic churns of butter were prepared in the Middle West. In each case each individual can of cream entering into the churn was classified and the percentage of each class

TABLE 5.—*Storage experiment*

SAMPLE NO.	0°F.		40°F.	
	MONTHS STORAGE	WIA	MONTHS STORAGE	WIA
		mg/100 g fat		*mg/100 g fat*
19	0	203	0	203
	4	204	4	207
	5	233	5	276
	22	277	22	462
8	0	135	0	135
	3	157	3	162
	5	273	5	449
	22	323	22	4427
29	0	411	0	411
	1	388	1	398
	5	400	5	1282
22	0	331	0	331
	1	354	1	347
	5	359	5	405
36	0	425	0	425
	2	401	2	421
	5	410	5	523
	22	458	22	7205
11	0	193	0	193
	2	230	2	235
	5	204	5	210
	22	258	22	295
5	0	104	0	104
	1	104	1	123
	5	109	5	78

in the churn was computed. Data are presented in Table 2 on vat acidity, percentage of the various classes of cream in each churn, WIA, and mold count (5). The samples are listed in the order of the increasing quantities of classes 1, 2, and 3 creams present.

The data show that butters in which no class 2 nor class 3 cream was present contained the lower quantities of WIA, and that when such decomposed cream was present the quantity of these acids was materially increased.

Portions of 14 of these authentic butters were stored at 40°F. for 75 days. There was no significant change in the WIA content during this storage period.

During the summer of 1945, 11 authentic churns of butter prepared under the same conditions as in 1944 were made in creameries located in Cincinnati, Ohio. Analytical data on these butters are given in Table 3. Again the higher figures for WIA were found in the butters churned from vat cream containing some cream classified as decomposed.

During the summer of 1946, 36 authentic churns of butter were similarly prepared in creameries located in the Middle West. Analytical data on these butters are given in Table 4.

TABLE 6.—*Analysis of sweet cream butter*

SAMPLE NO.	VAT ACIDITY	WIA	MOLD
	per cent as lactic	*mg/100 g fat*	*per cent*
1	0.12	95	0
2	0.12	89	0
3	0.07	109	0
4	0.10	100	0
5	0.11	112	0
6	0.11	98	0
7	0.13	80	0
8	0.19	78	0
9	0.15	88	0
10	0.17	110	0
11	0.18	104	0
12	0.15	142	0
13	0.15	76	0
14	0.13	84	0
15	0.18	226	0
16	0.11	114	0
17	0.15	122	0
18	0.13	128	0
19	0.17	121	0
20	0.16	131	0
21	0.13	115	0
22	0.14	90	0
23	0.12	101	0

Here again the higher values for WIA were found in the butters churned from vat cream containing some cream classified as decomposed.

Portions of 7 of the 1946 authentic butters were stored at 0° and 40°F. for periods up to 22 months. WIA were determined at different storage periods, as shown in Table 5.

With the exception of sample 8, there was no significant change in the

WIA content of any of these butters after 5 and in some cases 22 months of storage at 0°F. There was some increase in this sample between the third and fifth months of storage and a further small increase after 22 months, at which time visible mold appeared. At 40°F large clumps of mold appeared in samples 8 and 29 after 5 months storage, and the WIA increased approximately 3 fold. There was no significant change in the WIA content of the other 4 butters after 5 months of storage at 40°F., but, there was a decided increase after 22 months storage at 40°F.

During the summer of 1947, 23 churns of butter were made from sweet cream on a commercial scale. Analyses of these butters are presented in Table 6.

TABLE 7.—*Distribution of WIA between butter and buttermilk*

CLASS OF CREAM	PRODUCT	TITRATABLE ACIDITY AS LACTIC	WIA
		per cent	*mg/100 g fat*
0	Cream	0.12	176
	Butter		158
	Buttermilk		20*
1	Cream	0.66	241
	Butter		219
	Buttermilk		24*
2	Cream	1.09	683
	Butter		700
	Buttermilk		38*
3	Cream	1.54	762
	Butter		741
	Buttermilk		74*

* Mg per 100 g buttermilk.

An experiment was conducted to show the distribution of WIA between butter and buttermilk. One hundred gallons of sweet unpasteurized milk from a single producer were separated, and titratable acidity and WIA were determined on the cream. About 2½ gallons were churned, and WIA was determined in the butter. The balance of the cream was allowed to stand at room temperature (87 to 95°F.) with stirring each day. When Class 1 stage was reached it was again sampled for analysis and about 2½ gallons were neutralized to an acidity of approximately 0.2 per cent as lactic, pasteurized, and churned. This procedure was repeated when the cream reached the Class 2 and 3 stages. The analytical data are given in Table 7.

Most of the WIA were retained in the butter, very little being found in the buttermilk. The sum of the WIA in the butter and buttermilk is of

TABLE 8.—*WIA in cream*

SAMPLE NO.	ORGANOLEPTIC CLASSIFICATION	ACIDITY AS LACTIC	WIA
		per cent	*mg/100 g "as is"*
1	0	0.14	25
2	0	0.15	46
3	0	0.13	40
4	0	—	40
5	0	0.78	44
6	0	0.69	68
7	2	0.88	584
8	2	1.73	323
9	2	1.18	53
10	2	1.51	121
11	2	1.14	179
12	2	1.37	503
13	2	0.95	148
14	2	0.97	477
15	2	0.59	176
16	2	1.17	254
17	2	1.17	26
18	2	1.63	122
19	2	1.71	377
20	2	0.82	27
21	2	1.37	113
22	2	1.09	94
23	2	0.86	148
24	2	1.15	141
25	2	1.39	122
26	2	0.68	195
27	2	0.95	222
28	2	1.10	837
29	3	1.55	496
30	3	0.68	142
31	3	1.55	55
32	3	—	190
33	3	1.53	161
34	3	1.13	207
35	3	1.66	396
36	3	0.92	275
37	3	0.92	192

the same magnitude as that in the cream from which the butter was made.

During the investigation in the summer of 1946 a number of individual cans of cream were organoleptically classified and samples were analyzed for titratable acidity and WIA. The data are given in Table 8.

The creams classified as 0 contained small quantities of WIA. Thirty-one cans were classified as either 2 or 3. Twenty-seven of these contained materially larger quantities of WIA than were found in the 6 cans of sound cream. Only 4 of them contained WIA in an amount comparable to that found in the 6 cans of 0 class cream.

SUMMARY

Data are presented on the determination of WIA in 90 authentic churns of butter. When decomposed cream was present in the churn more WIA were found in the butter, in most cases, than in the butter churned from cream containing no decomposed material. It was shown that WIA in cream, for the most part, are retained in the butter on churning. Individual cans of cream classified as being decomposed usually contained much larger quantities of WIA than cans classified as satisfactory for butter making. There was in general little or no increase in WIA in butters stored at 0°F. for 5 months. Except in those cases in which visible mold appeared, there was no significant change in WIA in butters stored for 5 months at 40° F.

REFERENCES

(1) Hillig, F., and Knudsen, L., "Determination of Volatile Fatty Acids," *This Journal*, **25**, 176 (1942).

(2) Hillig, F., "Effect of Neutralization on Sour Milk in the Manufacture of Dried Skim Milk," *This Journal*, **25**, 253 (1942).

(3) Hillig, F., "Determination of Water-Insoluble Fatty Acids in Cream and Butter," *This Journal*, **30**, 575 (1947).

(4) Vandaveer, R. L., and Wildman, J. D., "Studies on the Mold Mycelia Count of Butter," *This Journal*, **23**, 693 (1940).

(5) *Methods of Analysis, A.O.A.C.*, 6th Ed., 1945, sec. 42.20.

VOLATILE ACIDS IN CREAM AND BUTTER*

PART I. THE DEVELOPMENT OF BUTYRIC ACID DURING THE PROGRESSIVE DECOMPOSITION OF CREAM

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PART II. BUTYRIC ACID IN COMMERCIAL CREAMS AND BUTTERS

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INTRODUCTION

The development of acidity in raw cream as it ages is universally known. The individual volatile acids formed during the progressive chemical changes in cream, inoculated microbially through normal handling, and held under controlled conditions of time and temperature, were investigated. Preliminary experiments indicated that some acetic acid is usually formed along with the lactic acid when cream sours spontaneously. Further, the retrogressive changes following the initial souring often result in the formation of butyric acid, occasionally as much as 0.5 per cent, accompanied by the development of objectionable organoleptic characteristics. The presence of butyric acid, in milk which has fermented, has been previously observed (1). Therefore the significance of butyric acid as an indication of decomposition in cream in controlled experiments and in commercial authentic creams, and the butter made therefrom, was studied.

PART I. THE DEVELOPMENT OF BUTYRIC ACID DURING THE PROGRESSIVE DECOMPOSITION OF CREAM

Raw, unpasteurized creams, with no micro-organisms present other than those introduced in good sanitary practice, were held 2–3 weeks at three temperatures: (*1*) in a 32°C. incubator, (*2*) at room temperature (25–27°C.), and (*3*) at a constant 20°C. temperature. Samples were withdrawn at frequent intervals for analysis. Lactic acid and lactose were determined by A.O.A.C. methods (2) as directed in sections **22.8** and **22.24**, respectively. At the beginning of these investigations, the method (3) then available for the individual determination of volatile acids when present together was used. This method, and a modification (4) used later, consists essentially of distilling the acids at constant volume at a uniform rate. The earlier computations were made by the procedure of Dyer (5). Later the Hillig and Knudsen (4) procedure was used. The published directions for the preparation of the sample (6) were adapted to cream and butter in the following manner:

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† W. B. White, Chief.

Preparation of butter sample.—Weigh 50 g into each of two centrifuge bottles, and proceed as directed in the method for water-insoluble acids in cream and butter (6), beginning with the words "Add NaOH" and continuing through the second washing with mixed ethers for removal of fat. Then remove residual ether from bottles by evaporation on steam bath, transfer contents of both to a single 200 ml volumetric flask with H_2O, add 1 ml H_2SO_4 (1+1) and 10 ml of 10% sodium tungstate soln. Make to mark, shake and filter. Transfer 150 ml of filtrate to distillation flask.

Preparation of cream sample.—Weigh 100 g into a 250 ml volumetric flask, add 100 ml of water and 2 ml of sulfuric acid (1+1), and mix, avoiding vigorous shaking. Add 15 ml of 10% sodium tungstate soln, make to mark, shake and filter through a rapid flowing filter paper. Transfer 150 ml of filtrate to distillation flask.

The changes in the constituents of the creams at the three different incubation temperatures are illustrated by Figures 1, 2, and 3, respectively. As would be expected, the lactose decreased throughout the experiments, since micro-organisms in milk tend to attack carbohydrates first. Especially at the higher temperatures, the disappearance was more pronounced near the beginning, at which time lactic acid was formed more rapidly.

With some creams, the rate of formation of lactic acid was later retarded, the amount in creams 1 and 2 (Fig. 1) even decreasing for a few days. This retardation was accompanied in these creams by a sudden, marked increase in volatile acids. These concurrent changes which occurred at an earlier period at the higher temperatures suggest that the volatile acids may have been formed at the expense of the lactic acid.

Butyric acid, as determined from the distillation constant (percentage of total volatile acid in the first 100 ml of distillate), was the principal volatile acid formed during this upsurge. In the earlier stages this acid was not detectable, the chief volatile acid then being acetic.

At the time butyric acid appeared, the creams were in violent fermentation. A disagreeable flavor, such as would lead the housekeeper to regard it as unfit or inedible cream, had developed. In some creams the flavor resembled that of plain sour cream to which butyric acid had been added. In others it resembled that of creams often described by cream graders in the industry as "cheesy." At this stage, the cream had separated into a dry, friable, spongy layer on the surface with a serum layer underneath similar in some respects to a yeasty type of fermentation, except that the latter is usually slimy rather than friable.

Although lactose appeared the logical source of the butyric acid formed as a result of microbial activities in cream, the butterfat is a potential source, since the butyric acid present as a glyceride could be liberated on hydrolysis by lipolytic enzymes. To check this possibility, after neutralization with sodium bicarbonate to about 0.25 per cent acidity as lactic,

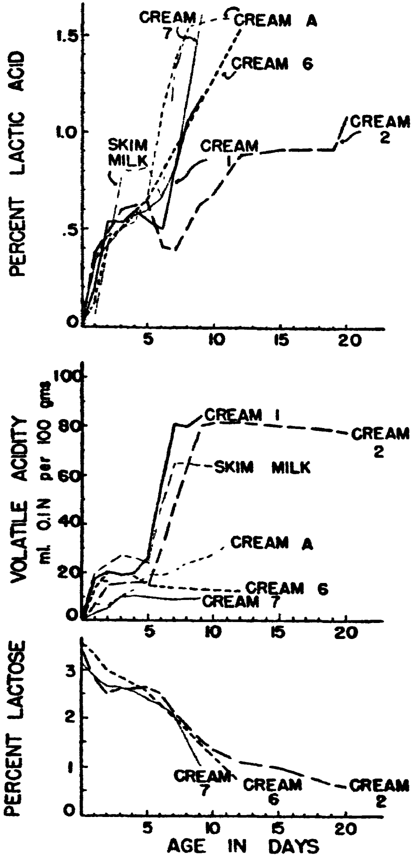

Fig. 1.—Incubated at 32°C.

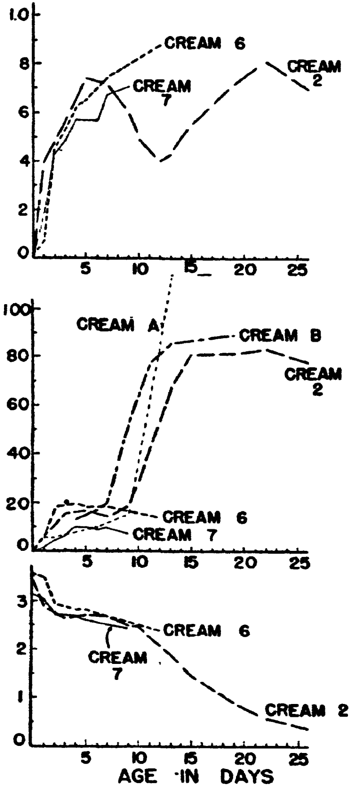

Fig. 2.—Incubated at 25–27°C.

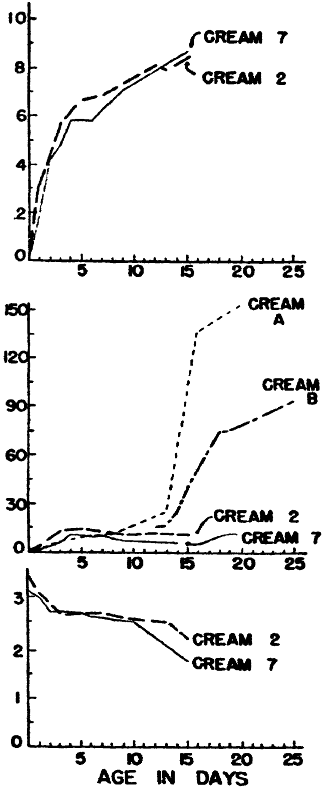

Fig. 3.—Incubated at 20°C.

the creams were churned and the Reichert-Meissl number of the fat from each butter was determined. Further, the distillation constant (percentage of total acids distilled in the first 100 ml of distillate) of the Reichert-Meissl acids was determined by the original method, after transfer of the titrated acids to the distillation flask. Since the total acids in the distilling flask were known, the use of equations was unnecessary.

TABLE 1.—*Reichert-Meisel numbers of butter*

AGE OF CREAM IN DAYS	CREAM 1		CREAM 2		CREAM 5		CREAM 7	
	R-M NO.	DIST. CONST.	R-M NO.	DIST. CONST.	R-M NO.	DIST. CONST.	R-M NO.	DIST. CONST.
Fresh	31.7	82.2	31.2	81.9	30.1	77.8	29.5	81.4
3	31.1	82.5						
5	31.4	81.7	31.1	80.9				
6							30.0	80.5
8	31.6	81.7						
10							29.3	81.0
12					30.0	77.8		
13	31.1	81.5						
14	30.7	80.6						

The results in Table 1 show that both the Reichert-Meissl number and the distillation constant of the Reichert-Meissl acids remained unchanged throughout the progressive decomposition of cream. The butyric acid glycerides in butterfat are significant contributors to the Reichert-Meissl number, and if an appreciable portion of them had been hydrolyzed the number would have been noticeably changed.

In order to test the possibility that the free butyric acid in the cream might have dissolved in the fat in just the right amount to maintain a constant Reichert-Meissl number, some of the butter was neutralized with sodium hydroxide, extracted 3 times with warm water, the residue dried with sodium sulfate, the fat extracted with petroleum ether and dried in a boiling water bath under vacuum. Treated in this manner it was found that there was no change in the Reichert-Meissl values, or in the distillation constant of the Reichert-Meissl acids, of the butter churned from the creams at various stages of incubation.

Further evidence on the source of the butyric acid was obtained on whole and skim milks. Typical results are illustrated by the skim milk curves in Figure 1. The 0.57 per cent of butyric acid found after six days' incubation at 32°C., as determined by the modified distillation method, (4) obviously could not have come from the milk fat. The skim milk contained 0.1 per cent of fat and, as butterfat contains about 3 per cent of combined butyric acid, the maximum theoretical amount from this source would have been only .003 per cent. Whole milks developed similar concentrations of butyric acid, whereas the total of this acid from the fat of a 4 per cent milk could be only 0.12 per cent.

By postulating that lactose on fermentation will produce 4 molecules of lactic acid, that 1 molecule of butyric acid will be formed from 2 molecules of lactic acid through a series of intermediate reactions, and that 1 molecule of acetic acid will be formed from one molecule of lactic acid, one may test more directly the hypothesis that the volatile acids are formed primarily from lactose. Acetic and butyric acids (calculated from the data used in preparing Fig. 1) have been converted into their assumed equivalent in lactic acid, and these amounts added to the lactic acid remaining as such in the cream. This sum has then been compared with the actual quantity of lactose lost. In Table 2 the results obtained in Cream 2 incubated at 20°.C have been so treated. Similar results were obtained on the same cream incubated at 25–27°C. and at 32°C.

TABLE 2.—*Comparison of loss in lactose and gain in lactic acid*
Cream incubated at 20°C.

AGE OF CREAM DAYS	GAIN IN LACTIC ACID	GAIN IN LACTIC ACID COMPUTED FROM ACETIC ACID	GAIN IN LACTIC ACID COMPUTED FROM BUTYRIC ACID	TOTAL GAIN IN LACTIC ACID	LOSS OF LACTOSE
1	0.308	0.042	—	0.350	0.35
3	0.563	0.118	—	0.681	0.78
5	0.667	0.124	—	0.791	0.77
7	0.685	0.109	—	0.794	0.77
9	0.737	0.102	—	0.839	0.87
12	0.804	0.072	0.074	0.950	0.94
13	0.779	0.051	0.095	0.925	0.93
14	0.817	0.041	0.112	0.970	1.10
15	0.840	0.033	0.131	1.004	1.19

The loss in lactose in all cases closely approximated the "total gain in lactic acid." Thus all the available evidence indicates that the butyric acid found in these creams was produced from lactose through lactic acid as an intermediate step.

To further verify the presence of butyric acid, a relatively large amount of total volatile acids distilled from Cream 2 of Figure 1 were redistilled, 100 ml distillates collected, and each titrated, following the method previously used to determine the highest volatile acid in a mixed distillate (7). From the data, Curve 1 of Figure 4 was drawn. The first 100 ml of titrated distillate obtained in preparing Curve 1 were acidified, transferred to the distilling flask and, various portions of distillate were collected to prepare Curve 2. The same procedure was followed in preparing Curve 3. Another curve was prepared, but since it coincided with Curve 3, it is not shown. Curve 3 falls below the line given by propionic acid similarly distilled and approaches, but never crosses, the line given by butyric acid.

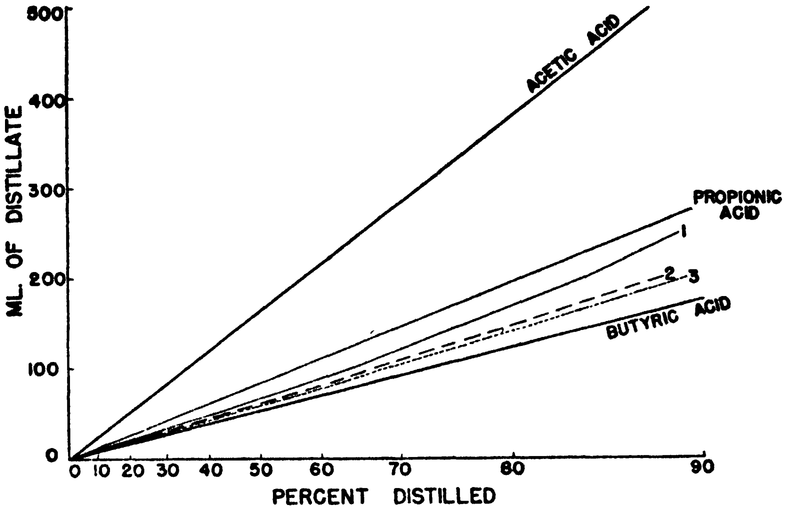

Fig. 4—Fractionation of volatile fatty acids from cream.

It may thus be concluded that butyric acid was the highest detectable volatile acid present in this sample.

PART II. BUTYRIC ACID IN COMMERCIAL CREAMS AND BUTTERS

In the studies described in Part I, it was observed that butyric acid when present in cream, carries over into the hand-churned butter. In order to test this observation on butters more closely approximating the commercial article, a series of small churnings were made, using an electrically driven, roller type churn of 12 gal. capacity. In each case about 4 gallons of cream, all from one producer, were neutralized, pasteurized, and churned, simulating as near as possible the regular commercial process in the details of churning, washing, working, etc. The creams were classified according to the proposed system of Vanderveer and Wildman (8).*

Butyric and propionic acids were determined in these butters by a chromatographic procedure (9). The results are given in Table 3, including grade of cream in the churn and the water-insoluble acids (WIA). A small and variable, but substantial, portion of the butyric acid, when present, usually remained in the butter. Propionic acid, however, was retained in only two of the butters made from creams containing it. This acid presumably was lost in the buttermilk and wash water.

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* See also *This Journal* (p. 743).

No determinable quantity of butyric acid was found in creams suitable for buttermaking. In the creams classed as 2 and 3, both butyric and propionic acids were found—in some cases in rather large quantities.

TABLE 3.—*Distribution of butyric and propionic acids between cream and butter*

SAMPLE NO.	CREAM				BUTTER		
	ORGANOLEPTIC CLASS	ACIDITY AS LACTIC	BUTYRIC ACID	PROPIONIC ACID	WIA	BUTYRIC ACID	PROPIONIC ACID
		per cent	*mg/100 g fat*	*mg/100 g fat*	*mg/100 g fat*	*mg/100 g fat*	*mg/100 g fat*
1	0	0.12	0	0	100	0	0
2	1	0.71	0	0	159	0	0
3	1	0.72	0	0	137	0	0
4	1	1.36	0	0	130	0	0
5	2	1.01	31	5	329	0	0
6	2	1.52	23	3	909	6	0
7	2	0.55	38	4	1362	4	0
8	2	0.88	40	6	1099	4	0
9	2	1.09	171	17	6573	40	0
10	2	1.43	53	6	1517	2	0
11	3	1.44	145	4	694	9	4
12	3	2.12	27	3	1435	0	0
13	3	2.46	176	15	918	4	3

In the companion study on WIA in creams and butters in 1946 (*This Journal*, p. 744), butyric acid was later determined in subdivisions from 37 cans of the cream. The creams were classified and sampled at the creamery, and represented either direct deliveries to the creamery by the producer, or cream picked up by a route driver and delivered to the creamery. The results are given in Table 4, and for convenience, the organoleptic classifications and WIA are repeated. Organoleptically these creams were typical examples of Classes 0, 2, and 3 as encountered in the industry. Class 2 creams had a flavor that is referred to in the industry as "slightly cheesy," or "cheesy." The flavor of the Class 3 creams was either "cheesy" or putrid. Such creams are considered decomposed.

The authentic commercial butters from the WIA studies (*This Journal*, p. 739) of 1945 and 1946, were also analyzed for butyric acid. The results are given in Table 5 with the classification of the creams in each churn. The results for WIA and mold are again included for convenient comparison.

From Table 5 it is apparent that butter churned from vat cream containing some Class 2, or Class 2 and 3 cream, frequently contains butyric acid and usually high WIA or mold count as well.

TABLE 4.—*Analysis of individual cans of cream—Summer, 1948*

SAMPLE NO.	ORGANOLEPTIC CLASSIFICATION	TITRATABLE ACIDITY AS LACTIC	WIA	VOLATILE ACIDS	
				BUTYRIC	PROPIONIC
		per cent	*mg/100 g*	*mg/100 g*	*mg/100 g*
1	0	0.14	25	0.0	0.0
2	0	0.15	46	0.0	0.0
3	0	0.13	40	0.0	0.0
4	0	—	40	0.0	0.0
5	0	0.78	44	0.0	0.0
6	0	0.69	68	0.0	0.0
7	2	0.88	584	9.6	0.0
8	2	1.73	323	27.4	0.0
9	2	1.18	53	3.4	0.0
10	2	1.51	121	26.6	9.5
11	2	1.14	179	50.2	18.5
12	2	1.37	503	16.5	0.0
13	2	0.95	148	63.9	0.0
14	2	0.97	477	10.7	0.0
15	2	0.59	176	16.5	0.0
16	2	1.17	254	0.0	0.0
17	2	1.17	26	0.0	0.0
18	2	1.63	122	48.2	0.0
19	2	1.71	377	56.2	11.3
20	2	0.82	27	0.0	0.0
21	2	1.37	113	12.2	0.0
22	2	1.09	94	6.7	0.0
23	2	0.86	148	6.7	0.0
24	2	1.15	141	53.2	7.0
25	2	1.39	122	36.2	3.6
26	2	0.68	199	49.4	0.0
27	2	0.95	222	8.4	0.0
28	2	1.10	837	28.7	2.1
29	3	1.55	496	160.7	62.0
30	3	0.68	142	94.9	0.0
31	3	1.55	55	3.8	5.2
32	3	—	190	20.7	0.0
33	3	1.53	161	303.6	0.0
34	3	1.13	207	122.8	46.5
35	3	1.66	396	37.1	0.0
36	3	0.92	275	122.4	46.3
37	3	0.92	192	112.5	6.5

TABLE 5.—*Authentic butters*

SAMPLE NO.	VAT ACIDITY AS LACTIC	CLASSES OF CREAM IN CHURN				W.I.A.	MOLD	BUTYRIC ACID
		0	1	2	3			
1945	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*mg/100 g fat*	*per cent*	*mg/100g fat**
1		100				50	18	0
2		82	18			145	74	0
3		66	34			140	64	0
4		61	39			154	40	0
5		34	66			104	44	0
6		76	23	1		61	20	0
7			96	4		163	76	0
8		2	88	10		315	64	4
9		5	73	22		374	82	3
10			71	27	2	543	82	6
11			61	30	9	565	96	5
1946								
1	0.12	100				76	0	0
2	0.17	100				93	0	0
3	0.15	100				104	0	0
4	0.38	55	45			159	2	0
5	0.50		100			100	1	0
6	0.68		98	2		133	21	0
7	0.81	11	87	2		209	51	0
8	0.90	4	94	2		200	37	3
9	0.82	6	92	2		261	53	2
10	0.87	4	93	3		150	10	3
11	0.95	13	84	3		273	86	7
12	0.85		97	3		209	79	0
13	0.80	2	94	4		203	68	5
14	0.57		95	5		128	21	0
15	1.14		94	6		315	76	5
16	0.85	10	84	6		331	79	3
17	0.94		93	7		394	76	4
18	0.45	2	88	10		201	2	0
19	0.80		89	11		269	17	0
20	0.81		84	16		371	5	5
21	0.92		80	20		231	81	2
22	0.77		76	24		248	21	0
23	1.15		90	9	1	411	93	3
24	1.00		83	16	1	350	24	2
25	0.58		90	8	2	260	15	3
26	1.50		66	32	2	381	2	0
27	1.22		72	25	2	448	92	7
28	1.05		67	19	14	425	95	2

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* The revised distillation method (4) was used on the 1945 samples and the chromatographic method (9) on the 1946 samples.

A test of the application of the butyric acid procedure to commercial butters was made on 23 samples purchased on the open market. The results are given in Table 6, along with results for WIA and mold. Butyric was absent in many of those samples containing the lower amounts of WIA.

TABLE 6.—*Analysis of market butter*

SAMPLE NO.	WIA	BUTYRIC ACID	MOLD
	mg/100 g fat	*mg/100 g fat*	
1	1050	28	64
2	501	23	58
3	733	11	24
4	464	11	22
5	599	13	26
6	969	19	44
7	238	3	88
8	243	3	82
9	264	3	50
10	293	3	50
11	320	3	52
12	251	none	40
13	198	4	87
14	600	16	44
15	964	20	42
16	343	8	18
17	450	9	22
18	99	none	0
19	106	none	0
20	174	none	4
21	181	none	2
22	541	15	22
23	593	15	28

In addition butyric acid was determined on 23 samples of authentic commercial sweet cream butters and no determinable quantities were found in any instance.

SUMMARY

Progressive decomposition experiments on cream show that butyric acid is frequently produced in cream when it reaches that stage of deterioration where it is unfit for human consumption. Results indicate that the butyric acid probably results from the breakdown of the lactose, through lactic acid as an intermediate step, and not from the hydrolysis of the fat and accompanying liberation of the combined butyric acid in the glycerides. When the acid is present in cream, some is usually carried over into the butter made therefrom.

The proportion so carried over is not important. So long as the amount is within the limit of detection by the method, its mere presence in the finished product is an indication that some decomposed cream was present in the vat mixture from which the butter was churned. No determinable quantity of butyric acid is present in butter churned from sound cream, nor was it found in commercial sweet cream butter.

Grateful appreciation is extended to S. W. Ahlmann, of the Cincinnati Station of the Food and Drug Administration, for his work in classifying all of the commercial creams used in this investigation. Also to those who made the mold counts, especially, W. J. McCarthy of Cincinnati Station, by whom the majority of counts were made. Thanks are also due to W. I. Patterson and H. A. Lepper for their suggestions and assistance during the development of this study.

REFERENCES

(1) Rogers & Associates. "Fundamentals of Dairy Science," 2nd Ed., p. 316 (1935).

(2) *Methods of Analysis*, 6th Ed., 1945.

(3) Clark, E. P., and Hillig, F., "Concerning the Dyer Method for the Identification and Determination of Volatile Fatty Acids," *This Journal*, **21**, 684 (1938).

(4) Hillig, F., and Knudsen, L. F., "Determination of Volatile Fatty Acids," *This Journal*, **25**, 176 (1942).

(5) Dyer, D. C., "A New Method of Steam Distillation for the Determination of the Volatile Fatty Acids," *J. Biol. Chem.*, **28**, 445 (1917).

(6) Hillig, F., "Water-Insoluble Acids in Cream and Butter," *This Journal*, **30**, 580 (1947).

(7) Hillig, F., and Clark, E. P., "A Chemical Procedure for Evaluating Spoilage in Canned Fish," *This Journal*, **21**, 688 (1938).

(8) Vandaveer, R. L., and Wildman, J. D., "Studies on the Mold Mycelia Count of Butter," *This Journal*, **23**, 693 (1940).

(9) Ramsey, L. L., and Patterson, W. I., "Separation and Identification of the Saturated Fatty Acids (C_1 to C_4)" *This Journal*, **28**, 644 (1945).

DETERMINATION OF 2-AMINOPYRIDINE IN ORANGES

By W. O. Winkler (Food Division,* Food and Drug Administration, Federal Security Agency, Washington, D. C.)

The chemical preservative 2-aminopyridine has been proposed as a fungicide for the control of stem-end rot in oranges. The suggested treatment was to immerse the fruit in a solution of the preservative, allow it to remain for a short time, and then rinse it in water.

Because of the toxic nature of 2-aminopyridine, and because it was suspected of penetrating into the flesh of the orange (a suspicion that was later confirmed), methods were investigated for its isolation and determination in treated oranges. Ether was selected as the most suitable solvent for isolating this preservative, which is characterized by its extreme solubility in many solvents. A dilute solution of 2-aminopyridine shaken with an equal volume of ether yields only 37 per cent of the chemical to the ether in the first shake-out; but four shake-outs with ether, or a continuous liquid extraction, proved to give satisfactory recoveries of the free base. However, this is true only of the free base; the acid compound is only slightly ether-soluble.

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* W. B. White, Chief.

Although most primary aromatic amines can be determined in small amounts by diazotization followed by a coupling reaction to yield an azo dye adapted to colorimetric measurement, 2-aminopyridine does not respond suitably to this series of reactions; and thus other means were sought for its determination. Methods considered for determining this preservative, after extraction with a selective solvent, were:

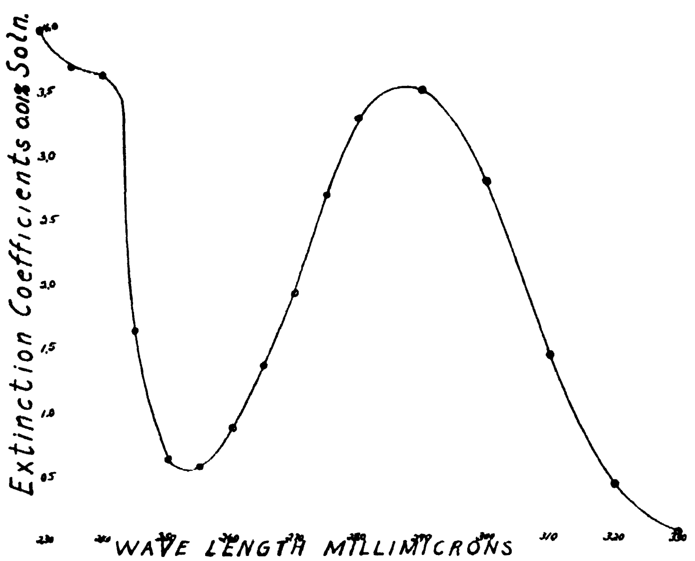

FIG. 1.—Ultra violet absorption spectrum of 2-aminopyridine.

(1) Direct titration using a suitable indicator and a standard buffer solution for comparison.

(2) Nitrogen determination of the extracted material. Both of these methods have the obvious disadvantage of non-specificity. However, in studies on orange juice, both appeared to give satisfactory results.

(3) In addition to the above two quantitative methods, the ultra-violet absorption spectrum of 2-aminopyridine, which shows a rather character-

istic maximum and minimum (Fig. 1), furnishes a qualitative confirmatory test capable of detecting many potential interferences.

(1) The reaction between 2-aminopyridine and hydrochloric acid is one of simple addition in equimolecular quantities but, like other reactions of weak bases with strong acids, it requires a rather low *p*H for completion. Since methyl red is usually employed for ammonia determinations, it was the first indicator tested. The results with this indicator were fairly consistent, but were always lower than the theoretical. Recoveries in water solution varied from 96 to 98 per cent of theoretical, and those in orange juice using 4 shake-outs with ether were from 91.5 to 93.4 per cent. Since the color change of methyl orange occurs at a considerably lower *p*H (3–4), titrations were made using this indicator. However, a few determinations showed that the results were too high. Therefore, bromcresol green was selected as an indicator having a *p*H range between that of methyl red and methyl orange.

By use of a series of buffer solutions differing by 0.2 *p*H unit, it was found that the color corresponding to *p*H 4.4 was the optimum end point for satisfactory results, when allowance was made for the water blank. Sample titrations were made to this *p*H by comparison with the standard buffer.

(2) The determination of 2-aminopyridine from its nitrogen content is very satisfactory, and if the result is confirmed by the titration procedure, there is a strong presumption that the isolated material is 2-aminopyridine. Since the ammonia produced gives just double the titration of the original 2-aminopyridine, it is apparent that we are dealing with a nitrogen compound which contains one basic nitrogen atom and one which is not basic. This fact limits considerably the number of possible interfering compounds.

Several semimicro methods of determining the nitrogen were tried to find the most satisfactory one. These were (a) digestion with sulfuric acid, sodium sulfate, and 30 per cent perhydrol (H_2O_2) (1); (b) digestion with sulfuric acid (containing sodium sulfate) and perchloric acid; and (c) a modified Kjeldahl-Gunning-Arnold method using sulfuric acid, sodium sulfate and mercuric oxide catalyst (2). After the first trial or two, the first two procedures were abandoned in favor of the last one (c).

(3) If additional evidence is needed to confirm the identity of the material isolated, the extinction curve in the ultraviolet region of the spectrum obtained on the solution of the compound will supply such evidence. The ultraviolet extinction curve of many chemicals is quite specific and characteristic, and this appears to be the case with 2-aminopyridine (Fig. 1). The position of the maximum and minimum, and the values of the extinction coefficients at these points on the curve, are effective in establishing with finality whether the material analyzed is 2-aminopyridine.

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C.S.

DETERMINATION OF 2-AMINOPYRIDINE IN ORANGES

APPARATUS

Siphon.—Insert two bent glass tubes in a two-hole cork or stopper, one (the mouthpiece) terminating just below the stopper and the other (the siphon tube) long enough to reach the bottom of a centrifuge bottle when the cork with tubes is inserted in the mouth of the bottle. Attach another glass tube to the outside arm of the siphon tube by means of a flexible rubber tube. (The assembly is used to siphon the lower layer from a centrifuge bottle, and the rate of flow is controlled by squeezing the rubber connection.) Prepare a cap for the inner arm of the siphon by boring a hole of the same diameter as the tube part way through a small cork.

PREPARATION OF SAMPLE

Bisect 8–12 oranges and juice with a reamer. Strain the juice to remove pulp and seeds and mix in a "blendor" for about 30 seconds. Transfer 150 ml (measured in graduate) of the mixed juice to a 250 ml centrifuge bottle. Add 50 ml of ether to the bottle, stopper, and shake well for 1–2 min. Place in centrifuge and whirl at about 1800 r.p.m. for 10 minutes. Cap the inner arm of siphon tube; insert it into the bottle, and lower it through the ether layer into the aqueous layer. Then with a glass rod push off the cap and remove the rod. Push the stopper into the mouth of the bottle and lower the end of the siphon to the bottom of the bottle. Start the flow of liquid by blowing, and filter the emerging liquid through a pledget of cotton in a short-stemmed funnel, into a 250 ml centrifuge bottle. Allow as much as possible of the lower layer to siphon over without molesting the ether layer, adjusting the flow, and finally stopping it, by squeezing the rubber connection in the siphon. Discard the ether layer.

To the siphoned liquid, add calcined powdered MgO at the rate of about 1 g per 100 ml. Stir with a glass rod and test at intervals with universal indicator paper.[1] When the liquid reaches a *p*H of about 7.5–8 (indicator paper same color as given by standard buffer soln at *p*H 7.6), add ca a heaping teaspoonful of filtercel, stopper, shake to disperse the filtercel, and immediately centrifuge for 10 minutes. Decant off the supernatant liquid into a 500 ml suction flask, stopper flask, attach to vacuum and evacuate slowly for several minutes to remove most of ether. (Momentarily let in air when the liquid threatens to froth out of the flask.) Proceed with the extraction of the 2-aminopyridine by method A or B.

REAGENTS

(1) *Washed ether.*—Shake 500–600 ml portion of ethyl ether with two 225 ml portions of water in a separatory funnel. Draw off water each time and filter ether through paper.

(2) *Standard 0.02 N hydrochloric acid.*

(3) *Standard 0.02 N sodium hydroxide.*

(4) *Bromcresol green indicator—0.1% soln.*—Rub 0.1 g of the solid with 7 ml 0.02 *N* NaOH in a mortar and make to 100 ml with water.

(5) *Magnesium oxide.*—Fine powder.

(6) *Buffer mixture p*H *4.4.*—Prepare M/5 potassium acid phthalate and place 50 ml in 200 ml vol. flask, add 7.5 ml of 0.2 *N* NaOH, and dilute to 200 ml with distilled water.

(7) *2-aminopyridine.*—Recrystallize 2-aminopyridine of Eastman quality from a strong soln in CCl_4, cool, dilute with cold CCl_4, and filter with suction. Wash with cold CCl_4 and allow solvent to evaporate the residue. Dry the residue in vacuum desiccator over H_2SO_4.

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[1] Hydrion papers A and B were very satisfactory.

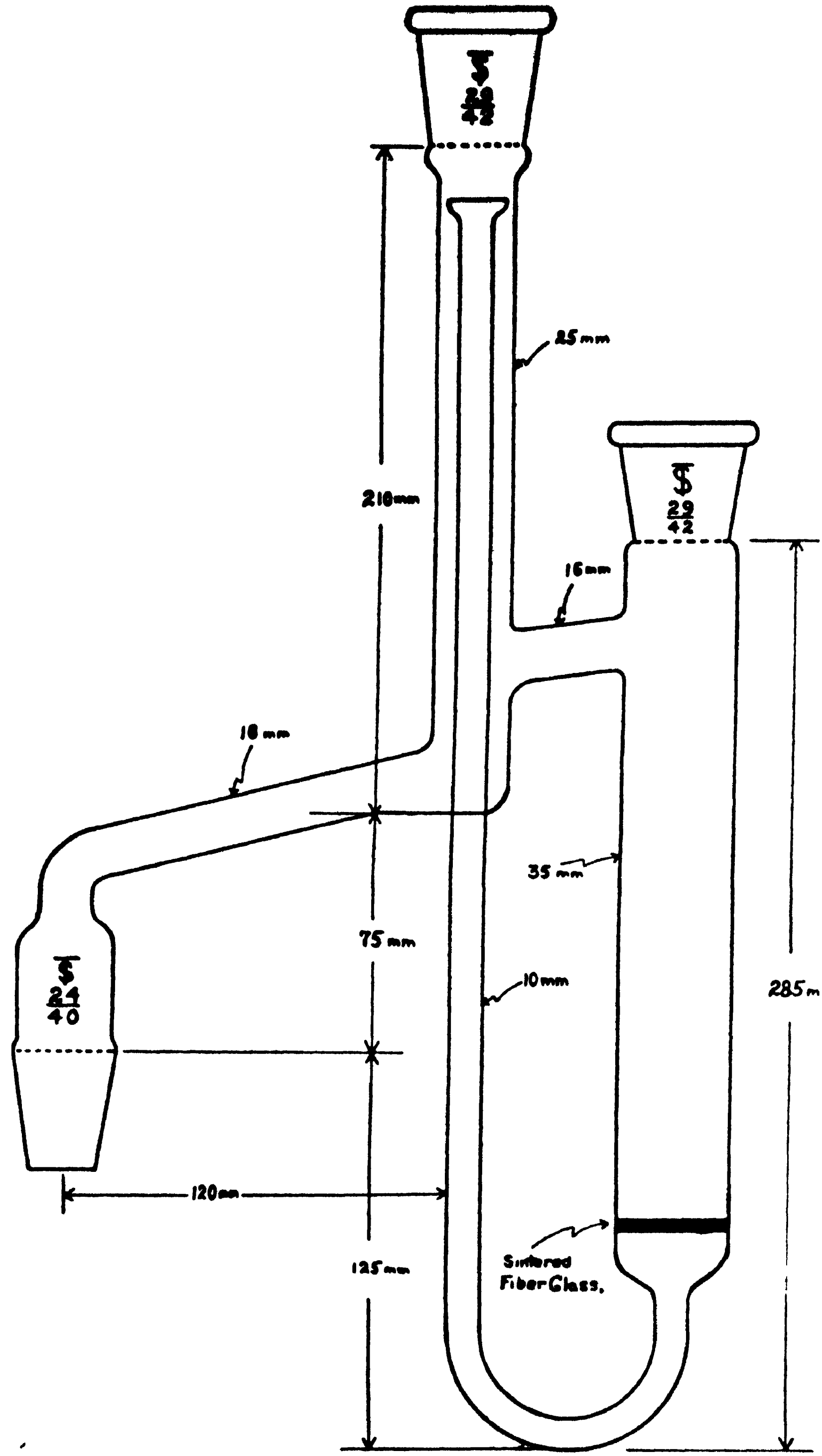

FIG. 2.—Continuous Extractor. Designed by John R. Matchett and Joseph Levine (Bureau of Narcotics, U. S. Treasury Department).

EXTRACTION METHOD A

Pipet 50 ml of the clear liquid into a 250 ml separatory funnel. Extract the liquid 4 times with 100 ml portions of washed ether, alternating between two funnels. Draw off all of the aqueous layer into the alternate funnel (remove liquid in stem by jolting), then pour off the ether extract through the mouth of the funnel, and filter it through a pledget of cotton (moistened with ether) into a 500 ml separatory funnel. After the first and second extractions, rinse the separatory funnel with 5 ml of ether and add the rinsings to the ether extracts.

EXTRACTION METHOD B

Place 100 ml of the clear liquid in a Palkin-type continuous extractor with sintered glass bubble plate,[3] or in the Matchett and Levine type extractor (Fig. 2). The sintered glass plate in either extractor should be of coarse porosity suitable for dispersing gases in liquids (diameter of openings about 150–190 microns). In order to avoid difficulty, the sample and ether should be added in the following manner: If the Palkin-type (sintered glass disc attached to the inner tube) is used, place the sample in the outer tube and fill the tube nearly to the overflow with washed ether. Fill the inner tube with ether by drawing it through the bottom in such a manner that there are no air pockets. Close the top so the tube remains full, and then lower it into the outer tube. (An alternate procedure is to lower it part way and pour ether in the top while it is being lowered to the bottom of the outer tube.)

If the Matchett and Levine type extractor (Fig. 2) is used, first fill the bottom of the U with ether until the level is above the sintered glass plate; then add the sample to the large arm containing the sintered glass plate while simultaneously pouring ether into smaller arm of the U to such a height that the aqueous layer does not pass below the sintered glass plate. After addition of the sample and solvent, close the opening of the large tube.

Extract for 100–120 minutes on an electric hot plate using a 250-ml Erlenmeyer flask containing 125 ml of ether as a receiver. Filter the extract through a pledget of cotton into a 500 ml separatory funnel. Rinse the extraction flask with two 25 ml portions of ether by stoppering and shaking each time, and filter into the ether extract. Finally, pour the contents of the extraction tube into another separatory funnel, draw off the aqueous layer, and filter the ether layer into the ether extract. Proceed with the determination.

DETERMINATION BY TITRATION

To the combined ether extracts in the separatory funnel, add about 40 ml of distilled water and 5–10 ml of 0.02 *N* HCl accurately measured (10 ml of 0.02 *N* HCl is equivalent to 18.82 mg of 2-aminopyridine). Shake well for about 1 minute, allow the layers to separate, swirl the funnel to loosen droplets on the sides, and draw off the lower aqueous layer into a 200 ml Erlenmeyer flask. Give the funnel a whirl, allow to settle, and draw off the aqueous liquid again. Add a second portion of 40–45 ml of distilled water to the funnel and shake well again. Draw off the aqueous layer and combine with the first aqueous extract in the flask. Again give the funnel a whirl, let stand, and draw off any additional aqueous liquid. If the qualitative spectrophotometric test is to be made, make the acid aqueous extracts to a volume of 110 ml and take a 100 ml aliquot for the quantitative determination by titration or nitrogen content. Reserve the 10 ml portion for the spectrophotometric test. Add 3 drops of bromcresol green indicator to the acid extracts, or aliquot, and titrate with standard NaOH (0.02 *N*) until the indicator turns blue. The end point, *p*H 4.4, has now been slightly passed. Bring the soln to *p*H 4.4 with 0.02 *N* HCl by titrating to the same color as a similar volume (about 110 ml) of the *p*H 4.4 buffer soln containing 3 drops of the indicator. Titrate 10 ml of the 0.02 *N* HCl (diluted to ca 100 ml volume with water) to *p*H 4.4 with the standard alkali, using the same indicator to obtain the acid equivalent of the alkali at *p*H 4.4. (The titrated soln may be reserved for a nitrogen determination.) Obtain the acid consumed by the sample by subtracting the acid equivalent of the alkali from the total acid used. From the volume of acid consumed, subtract a reagent blank of 0.08 ml for extraction method A, or 0.15 ml for extraction method B, to obtain the acid necessary to react with the 2-aminopyridine present.

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[3] The type used was similar to No. 1502 in Scientific Laboratory Glassware Catalog of Eck and Krebs, and that in General Catalog 40, Ace Glass, Inc. No. 6840.

Multiply the correct titer (in ml 0.02 acid) by the factor 1.98 to obtain mg of 2-aminopyridine in 50 ml of sample if extraction method A was used.[3] Multiply the corrected titer by the factor 1.882 to obtain mg 2-aminopyridine in 100 ml of sample if extraction method B was used. The values obtained must be multiplied by 1.1 if an aliquot of 100 ml from a total volume of 110 ml was used.

DETERMINATION BY NITROGEN CONTENT

Shake out the combined ether extracts once with about 40 ml of 0.02 *N* HCl, and twice with 40 ml portions of distilled water. (The titrated soln may also be used for the determination.) Add 4 ml conc. H_2SO_4 to the combined acid extracts, evaporate in a beaker (250–400 ml) to about 25–50 ml, and transfer to a 125 ml Erlenmeyer flask. Add to the soln 1.5 g anhydrous Na_2SO_4 and 0.1 g of HgO. Boil down on the hot plate under a hood until the water is removed and the white SO_3 fumes are produced. After the liquid has fumed for several minutes, insert a small short-stemmed funnel in the mouth of the flask. Continue the digestion for 30 min. after the yellow or brownish soln first produced becomes clear and colorless. Cool, and transfer the digest to a 300 ml Erlenmeyer flask with ca 140 ml of water. Add 15 ml of the 50% NaOH soln without mixing, then 5 ml of $Na_2S_2O_3$ soln, connect to the usual trap and condenser and distil.

Receive the distillate in a 200 ml Erlenmeyer flask containing a measured quantity (10–20 ml) of standard 0.02 *N* HCl and 5 drops of methyl red indicator.[4] Distil until about 100 ml of distillate is collected. Titrate the excess acid in the receiver with standard 0.02 *N* NaOH and determine the acid consumed by the NH_3 distilled. Conduct a blank determination on the reagents and subtract the amount of acid consumed in the blank from the titer of the sample. Each ml of 0.02 *N* HCl consumed is equal to 0.2801 mg of nitrogen, or 0.941 mg of 2-aminopyridine. Divide the amount found by 0.95 if extraction method A was used. If a 10 ml portion of the liquid from 110 total was reserved for the spectrophotometric test, multiply the result by the factor 1.1.

CONFIRMATORY SPECTROPHOTOMETRIC TEST

To confirm that 2-aminopyridine is the material being determined, place a portion of the reserved aliquot in a quartz cell and make readings in a spectrophotometer at intervals of 5 or 10 mμ in the ultraviolet region between 230 and 340 mμ. Use .02 *N* HCl, in a matched cell, as blank. From the concentration value of 2-aminopyridine, obtained either by titration or *N* determination, convert extinction readings at each wave length to corresponding coefficients expressed in terms of a .01% concentration and 1 cm cell ($E_{1\text{cm}}^{.01\%}$). Compare this extinction coefficient curve with one prepared with a dilute acid soln of pure 2-aminopyridine read in a similar cell. In the preparation of the standard curve it is best to use a standard soln containing 5 mg per 100 ml (.005%) and convert to standard conditions (.01%, above) by multiplying extinction values at each wave length by 2. The standard curve is shown in Fig. 1. It shows a minimum at about 253 mμ and a maximum at about 287 mμ.

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[3] The factor 1.98 is obtained by dividing 1.882 (the mg of 2-aminopyridine neutralized by 1 ml of 0.02 *N* HCl) by the factor 0.95, since only 95% of the chemical is recovered by the 4 extractions provided in the method.

[4] Methyl red indicator 0.1% soln. Dissolve 0.1 g of solid in 60 ml 95% alcohol and make to 100 ml with water.

RESULTS

Some results on titration of water solutions of 2-aminopyridine are given in Table 1.

TABLE 1.—*Titration of 2-aminopyridine with 0.02 N HCl to pH 4.4 using bromcresol green indicator*

PRESENT	FOUND	RECOVERY
mg	*mg*	*per cent*
25	24.9	99.6
25	25.16	100.6
25	24.99	100.0
25	25.03	100.1
25	24.92	99.7
10	9.95	99.5
10	9.95	99.5

It is apparent that this method is quite satisfactory as a titration procedure. The end point is evidently correct for stoichiometrically equivalent amounts of the acid and base.

Table 2 lists results on known amounts of 2-aminopyridine added to orange juice, with closely controlled *p*H of the extraction solution and titration to *p*H 4.4 using bromcresol green indicator.

TABLE 2.—*Titrametric determination of 2-aminopyridine extracted from orange juice by extraction method A*

SAMPLE	2-AMINO-PYRIDINE ADDED	NO. OF ETHER EX-TRACTIONS	HCl CONSUMED	2-AMINO-PYRIDINE FOUND IN EXTRACTS	RECOVERY IN EXTRACTS
	mg		*ml*	*mg*	*per cent*
1	22.73	4	11.52	21.68	95.4
2	47.62	4	24.01	45.18	94.9
3	47.62	4	23.93	45.04	94.6
4	20	4	10.14	19.08	95.4
5	20	4	10.12	19.04	95.2

It is evident that extraction method A gives consistent recoveries of 95 per cent.

Table 3 lists a few similar determinations made on orange juice using the continuous extractor.

The results show that a complete extraction of the total 2-aminopyridine is readily obtained using the continuous extractors with the fritted

TABLE 3.—*Titrametric determination of 2-aminopyridine extracted from orange juice by extraction method B*

ADDED	FOUND	RECOVERY
mg	*mg*	*per cent*
20.0	20.08	100.4
10.0	10.03	100.1
25	24.12*	96.48*
10	9.96	99.6

* Extraction with lactic acid type extractor (3). (No fritted glass bubbler.)

glass bubblers (Fig. 2). For the time specified, extraction is not quite as complete with the lactic acid type of extractor.

The amounts of 2-aminopyridine (in water solutions) calculated from the nitrogen content found by the three digestion procedures mentioned in the introduction, are given in Table 4.

TABLE 4.—*2-aminopyridine calculated from nitrogen content*

SAMPLE	PROCEDURE*	2-AMINO-PYRIDINE ADDED	NITROGEN FOUND	2-AMINO-PYRIDINE CALCULATED	RECOVERY
		mg	*mg*	*mg*	*per cent*
1	(a)	50	12.62	42.37	84.7
2	(b)	50	4.38	14.71	29.42
3	(c)	100	29.79	99.83	99.83
4	(c)	50	14.91	49.85	99.7
5	(c)	50	14.93	49.9	99.8
6	(c)	10	3.054	10.01	100.1

* (a) Digestion with H_2SO_4 and perhydrol.
(b) Digestion with H_2SO_4 and perchloric acid.
(c) Modified Kjeldahl-Gunning-Arnold method.

It is evident that 2-aminopyridine can be accurately determined from the nitrogen content by the modified Kjeldahl-Gunning-Arnold method. Digestion by the other procedures was not satisfactory.

Recoveries of 2-aminopyridine extracted from samples of orange juice by methods A and B are given in Table 5.

The results obtained by the nitrogen method are very satisfactory, and are probably more specific than by the direct titration method. Identical results by the direct method and by the nitrogen determination confirm the presence of an amino compound containing one nitrogen atom which reacts with mineral acid and one which does not so react. The most common food constituent which contains two atoms of nitrogen in the molecule, and which has a somewhat similar structure, is nicotinic acid amide. However, this substance is only sparingly soluble in ether and furthermore, does not titrate with mineral acid under the conditions specified.

TABLE 5.—*Determination of 2-aminopyridine in orange juice from nitrogen content*

SAMPLE	2-AMINO-PYRIDINE ADDED	EXTRACTION PROCEDURE USED	NITROGEN FOUND	2-AMINO-PYRIDINE FOUND IN EXTRACT	RECOVERY CORRECTION APPLIED WHEN EXTRACTION A USED
	mg		*mg*	*mg*	*per cent*
1	10	B	2.96	9.95	99.5
2	20	B	5.91	19.84	99.2
3	25	B*	7.22	24.25*	97.0*
4	20	A	5.64	18.94†	99.6

* A lactic acid type extractor (3) was used for this sample.
† Actual figure was divided by 0.95 since extraction method A was used.

SUMMARY

Two methods are presented for the determination of 2-aminopyridine in oranges. Both methods use ethyl ether to extract the chemical from the prepared sample, adjusted to a required *p*H. In the first method, the chemical is determined in the extract by direct titration to *p*H 4.4 using bromcresol green indicator. The nitrogen content of the extracted matter forms the basis for the second method. Data are presented which show both methods to be reliable and accurate.

REFERENCES

(1) *Ind. Eng. Chem., Anal. Ed.*, **17**, 734 (1945).
(2) *Methods ofAnalysis, A.O.A.C.*, Sixth Ed. (1945), sec. **226**, page 27.
(3) *This Journal*, **25**, 256 (1942).

DETERMINATION OF REDUCING SUGARS IN FOOD PRODUCTS

COMPARATIVE STUDY OF COLORIMETRIC METHODS

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INTRODUCTION

Colorimetric methods for the determination of dextrose in blood and urine have been widely used. Their application to food products has been rather limited. The Lewis-Benedict and Sumner methods have been used by several investigators in the analysis of dairy products. Jackson (1) has suggested that the Benedict copper method be used for small amounts of reducing sugars but supports his article by no analytical results.

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TABLE 1.—*Error caused when various amounts of dextrose were compared to A standard of 100 mg*

DEXTROSE	THEOR. READING	ACTUAL READING	DEXTROSE FOUND	ERROR
mg per 100 ml			*mg*	*per cent*
		Sumner Method		
300	6.67	6.80	294.1	1.97
200	10.00	9.96	200.8	0.40
150	13.33	13.36	149.7	0.20
100	20.00	19.98	100.1	0.10
75	26.67	26.82	74.6	0.57
50	40.00	40.38	49.5	1.00
		Kingsbury Method		
300	6.67	6.75	296.3	1.23
200	10.00	10.10	198.0	1.00
150	13.33	13.45	148.7	0.81
100	20.00	20.01	99.9	0.10
75	26.67	26.69	74.9	0.13
50	40.00	40.45	49.4	1.20
		Poe-Edson Method		
300	6.67	6.78	295.0	1.67
200	10.00	10.04	199.2	0.40
150	13.33	13.32	150.2	0.13
100	20.00	20.02	99.9	0.10
75	26.67	26.80	74.6	0.53
50	40.00	40.34	49.6	0.80
		Folin Method		
300	6.67	7.82	255.7	14.8
200	10.00	10.42	119.9	4.1
150	13.33	13.62	146.8	2.13
100	20.00	19.93	100.3	0.30
75	26.67	26.94	74.2	1.07
50	40.00	40.98	48.8	2.40
		Folin-Wu Method		
300	6.67	8.02	249.4	16.87
200	10.00	10.34	193.4	3.30
150	13.33	13.67	146.3	2.47
100	20.00	20.05	99.7	0.30
75	26.67	26.83	74.5	0.67
50	40.00	40.87	48.9	2.20
		Benedict Method		
300	6.67	7.53	265.6	11.47
200	10.00	10.21	195.9	2.05
150	13.33	13.54	147.7	1.53
100	20.00	20.04	99.8	0.20
75	26.67	26.84	74.5	0.67
50	40.00	40.73	49.1	1.80
		Lewis-Benedict Method		
300	6.67	7.30	274.0	8.66
200	10.00	9.74	205.3	2.65
150	13.33	13.58	147.3	1.80
100	20.00	19.92	100.4	0.30
75	26.67	26.89	74.4	0.88
50	40.00	40.54	49.3	1.20

The object of this investigation was to compare the various colorimetric methods as to their availability for use in analyzing syrups and fruit products. The authors (2) have reported the use of sodium 2,4-dinitrophenolate as the basis of a reagent for the determination of reducing sugars. For food products this method gave very satisfactory results when compared with the Munson and Walker method. In addition to the above colorimetric method, the following well known methods were studied: Lewis-Benedict (3–4); Folin-Wu (5); Benedict copper method (6); Folin (7); Benedict copper method, new (8); Folin, new (9); Folin-Wu, new (9); Sumner, new (10); and Kingsbury (11).

A number of investigators have found with certain colorimetric methods that the length of the color column of the unknown and standard should be nearly equal. If this condition is not fulfilled, the reading will not be proportional to the percentage of reducing sugar present. According to Rothberg and Evans (12), this condition is not true for the Folin-Wu method. Folin and Dennis (13) and Bierman and Doan (14) state that the same disadvantage is found with the picric acid method of Lewis and Benedict. Sumner (10), however, reports with his methods that readings of 1 mg. of dextrose in the standard would give proportional readings with 0.5 and 2 mg. in the unknown. Benedict (8) claims the same condition to exist with his method. In order to test each of the methods in this respect, various concentrations of dextrose varying from 0.05 to 0.3 per cent were compared with a 1 per cent standard by using each method. With the methods of Folin, Folin-Wu, Kingsbury, and Benedict, the standard and test solutions were diluted to one-fifth because of the intensity of color in the stronger solutions. The results of these comparisons are given in Table 1. Each result is an average of five readings.

In examining the data in the table, it may be noted that the Sumner, Kingsbury, and Poe-Edson methods gave readings which were directly proportional to the amount of dextrose present. The other methods gave results for the higher and lower amounts which were not proportional. For greater accuracy in all methods, it seems better to have the readings of known and unknowns as nearly equal as possible.

A number of syrups and sugars were analyzed by using several of the original colorimetric methods. The results are reported in Table 2. The results for the Munson and Walker (15) method are given for comparison. The data given in Table 2 indicate that all of the methods give fairly satisfactory results. The merits of each method will be discussed later. All data given in Tables 2 to 6, inclusive, are based on duplicate determinations. The percentages of sugar were calculated as dextrose and no method of clarification was used. In Table 6 the sucrose in all samples was inverted with HCl. All other samples were analyzed before inversion.

A number of the older methods were discarded, and a series of samples were run using the latest modification of each of the methods. These modifications included the new proposed method of Benedict (8), Folin (9), Folin-Wu (9), and Sumner (10).

The comparisons of these various methods together with the methods of Lewis-Benedict, Kingsbury, and Poe-Edson were then made by using other food products such as fruit juices, jellies and flavoring syrups. The results of these tests appear in Tables 3, 4, and 5. The data given in these tables confirm the previous results listed in Table 2. The sucrose in a

TABLE 2.—*Analysis of syrups for reducing sugars**

FOOD PRODUCT	METHOD					
	MUNSON-WALKER	POE EDSON	SUMNER	FOLIN	LEWIS-BENEDICT	BENEDICT
Pure maple	6.18	6.25	6.25	6.35	6.23	6.13
Pure maple	6.58	6.65	6.60	6.60	6.62	6.65
Pure maple	2.13	2.20	2.16	2.28	2.20	2.28
Pure maple	1.84	1.86	1.83	1.85	1.80	1.80
Pure maple	4.56	4.58	4.55	4.60	4.62	4.56
Pure maple	1.92	2.12	2.12	2.03	2.10	2.03
Pure maple	2.58	2.68	2.50	2.65	2.66	2.72
Maple cake	6.45	6.50	6.51	6.61	6.58	6.49
Maple cake	5.61	5.68	5.68	5.75	5.68	5.71
Maple cake	6.58	6.62	6.63	6.60	6.53	6.60
Maple cake	5.72	5.66	5.70	5.66	5.61	5.76
Maple cake	0.83	0.95	0.90	1.09	0.93	1.02
Maple and cane	2.09	2.22	2.22	2.17	2.20	2.25
Cane and maple	2.56	2.63	2.60	2.63	2.58	2.54
Cane and maple	2.56	2.63	2.67	2.65	2.61	2.65
Cane and maple	6.13	6.26	6.25	6.19	6.32	6.25
Cane and maple	4.53	4.53	4.58	4.50	4.46	4.49
Cane and maple	4.79	4.86	4.82	4.92	4.80	4.82
Cane and maple	1.61	1.66	1.58	1.60	1.53	1.69
Cane syrup	4.65	4.76	4.71	4.76	4.71	4.66

* Results expressed as percentage of dextrose.

TABLE 3.—*Analysis of fruit juices for reducing sugars**

Fruit Juice	Method							
	Munson-Walker	Poe-Edson	Sumner	Folin	Folin-Wu	Lewis-Benedict	Benedict	Kingsbury
Apple	9.60	9.66	9.64	9.75	9.71	9.71	9.78	9.71
White grape	13.44	13.61	13.64	13.65	13.59	13.59	13.53	13.60
Pineapple	8.96	9.14	9.09	9.14	9.06	9.01	9.11	9.01
Orange	5.12	5.12	5.07	5.05	5.09	5.11	5.05	5.03
Loganberry†	8.88	9.05	9.09	9.17	9.01	9.02	8.97	8.95
Loganberry†	22.36	22.42	22.32	22.44	22.35	22.35	22.48	22.26
Grapefruit†	30.92	31.06	31.00	31.12	31.04	31.12	30.98	31.07

* Results expressed as percentage of dextrose.
† Concentrated, sweetened juices.

TABLE 4.—*Analysis of jellies and jams for reducing sugars**

Food Product	Method							
	Munson-Walker	Poe-Edson	Sumner	Folin	Folin-Wu	Lewis-Benedict	Benedict	Kingsbury
Apple jelly	16.61	16.55	16.51	16.45	16.51	16.66	16.69	16.53
Grape jelly	23.92	23.90	23.87	23.80	23.91	23.90	23.97	23.84
Raspberry jelly	17.12	17.01	17.10	17.00	17.18	17.01	17.14	17.18
Currant jelly	48.62	48.76	48.84	48.66	48.74	48.76	48.97	48.91
Plum jam	48.90	48.98	48.96	48.73	48.83	49.98	48.73	48.77
Strawberry jam	48.81	48.88	48.87	48.63	48.71	48.76	48.96	48.81
Pineapple-apricot jam	12.05	12.03	11.95	12.00	12.13	12.03	11.83	12.19
Grapefruit marmalade	26.16	26.27	26.22	26.22	26.19	26.27	26.10	26.09
Peach-pineapple jam	19.87	20.08	20.00	20.07	19.98	19.96	20.03	19.78

* Results expressed as percentage of dextrose.

TABLE 5.—*Analysis of flavoring syrups for reducing sugars**

Flavoring Syrup	Method							
	Munson-Walker	Poe-Edson	Sumner	Folin	Folin-Wu	Lewis-Benedict	Benedict	Kingsbury
Grape	18.16	18.14	18.14	18.11	18.06	18.22	18.12	18.28
Cherry	48.13	48.25	48.09	48.21	48.37	48.29	48.04	48.22
Peach	29.02	29.06	29.01	28.91	28.82	28.93	28.97	29.07
Plum	35.93	36.13	36.09	36.15	36.27	36.19	36.11	36.07
Lemon	4.84	4.87	4.90	4.85	4.80	4.83	4.94	4.91
Orange	15.12	15.29	15.19	15.09	14.98	15.15	14.93	15.16
Grapefruit	18.16	18.29	18.28	18.21	18.26	18.25	18.12	18.26
Raspberry	14.92	14.97	15.13	15.04	14.81	14.99	15.01	15.04

* Results expressed as percentage of dextrose.

TABLE 6.—*Determination of reducing sugars* after inversion of the sucrose*

Food Product	Method							
	Munson-Walker	Poe-Edson	Sumner	Folin	Folin-Wu	Lewis-Benedict	Benedict	Kingsbury
Syrup	62.65	62.68	62.58	62.58	62.62	62.58	62.82	62.80
Syrup	70.55	70.72	70.42	70.77	70.77	70.42	70.48	70.67
Syrup	66.62	66.76	66.66	66.88	66.80	66.82	66.60	66.71
Syrup	66.82	66.86	66.76	66.72	66.82	66.90	66.92	66.60
Syrup	68.70	68.82	68.68	68.60	68.76	68.70	68.81	68.78

* Results expressed as percentage of dextrose.

number of food products was completely inverted by treating with hydrochloric acid. After neutralization, the reducing sugars were determined as dextrose by each of the newer methods. The results are listed in Table 6.

In food analysis, frequently it is necessary to clarify the sugar solution before the reducing sugar is determined. This process is especially used with molasses, jellies, and some fruit juices. The first test with clarifying agents was performed on two samples of a 1 per cent dextrose solution. One sample was treated with different clarifying agents and made up to volume. The second sample was untreated but made up to the same volume. The dextrose was determined by the different methods. The standard was set at 20 and the treated samples were read. The results are given in Table 7. As will be noted from these results, all of the clarifying agents except mercuric nitrate showed no effect on the accuracy of the seven different colorimetric methods used. These results confirm those reported by Klemme and Poe (16) on the use of clarifying agents in the determination of sugars in bacterial cultures. A sample of maple sugar syrup was used with several of the clarifying agents. The results with such agents are satisfactory, as is shown in Table 8. The values obtained with alumina cream are somewhat lower with several of the methods.

TABLE 7.—*Effect of clarifying agents on colorimeter reading**

Clarifying Agent	Method						
	Poe-Edson	Sumner	Folin	Folin-Wu	Lewis-Benedict	Benedict	Kingsbury
Norite	19.94	20.02	20.08	20.06	19.90	19.94	20.04
Alumina cream	20.04	19.96	20.03	20.07	20.23	19.90	20.07
Dry lead subacetate	20.04	20.04	19.94	20.02	20.05	20.07	20.02
Lead sub-acetate solution	20.06	20.07	20.02	20.06	19.96	20.04	20.07
Mercuric nitrate	Ppt.	Ppt.	17.94	17.82	16.17	16.17	Ppt.

* Readings after clarifying. Colorimeter reading of standard dextrose solution set at 20.00.

TABLE 8.—*Determination of reducing sugars* in maple syrup after different clarifying agents were used*

Clarifying Agent	Method							
	Munson-Walker	Poe-Edson	Sumner	Folin	Folin-Wu	Lewis-Benedict	Benedict	Kingsbury
Norite	2.81	2.88	2.85	2.84	2.89	2.82	2.87	2.83
Fullers Earth	2.79	2.86	2.89	2.80	2.83	2.80	2.87	2.80
Lead sub-acetate	2.76	2.85	2.82	2.86	2.81	2.86	2.82	2.85
Alumina cream	2.68	2.77	2.73	2 76	2.78	2.69	2.75	2.74
None	2.79	2.82	2.80	2.78	2.86	2.78	2.84	2.82

* Results calculated as percentage of dextrose.

According to Browne and Zerban (17), Bryan, Horne, and other workers report that reducing sugars, more especially levulose, are carried down or occluded in the basic lead acetate precipitate when such salts as tartrates, malates, phosphates, sulfates, etc. are present.

DISCUSSION

The various colorimetric methods which have been proposed for the estimation of reducing sugars in blood and urine have been used in the present research for the quantitative estimation of reducing sugars in food products. Apparently, it seems that all of these methods could be used for the quantitative estimation of reducing sugars in food products. On closer study, it will be seen that there are a number of disadvantages to be encountered when some of the methods originally proposed for urine and blood analysis are used.

In the first place, with all the methods, with the exception of those of Sumner, Lewis-Benedict, and Poe-Edson, the dilution factor may introduce an error. In all methods, with the exception of the Sumner and the Poe-Edson methods, it is necessary to have two stock solutions from which the working solution is prepared. The reagents for the two above-mentioned methods are each made up as one solution and both have been known to keep for several months. This condition is a distinct advantage in that if only a few determinations are to be made, the reagent can be kept for some time; whereas with some of the other methods the working solution deteriorates in a very short time.

With the Poe-Edson method, the solution after reduction may stand as long as twenty minutes without fading of the color, whereas with a number of the other methods the reading must be taken at once, because otherwise the fading of the color gives inaccurate results.

The use of most clarifying agents did not interfere with the accuracy of the different colorimetric methods.

CONCLUSIONS

(1) The standard colorimetric methods are satisfactory for the determination of reducing sugars in food products.

(2) The common clarifying agents, except mercuric nitrate, do not interfere with the determination of reducing sugars.

REFERENCES

(1) Jackson, R. F., "Report on Chemical Methods for Reducing Sugars," *This Journal*, **11**, 175 (1928).

(2) Poe, C. F., and Edson, F. G., "Determination of Reducing Sugars in Food Products. Proposed Colorimetric Method," *Ind. Eng. Chem.*, *Anal. Ed.*, **4**, 300 (1932).

(3) Lewis, R. C., and Benedict, S. R., "A Method for the Estimation of Sugar in Small Quantities of Blood," *J. Biol. Chem.*, **20**, 61 (1915).

(4) Willaman, J. J., and Davidson, F. R., "Some Modifications of the Picric Acid Method for Sugars," *J. Agric. Res.*, **28**, 479 (1924).

(5) Folin, O., and Wu, H., "I. A Simplified and Improved Method for the Determination of Sugar," *J. Biol. Chem.*, **41**, 367 (1920).

(6) Benedict, S. R., "The Estimation of Sugar in Blood and Normal Urine," *J. Biol. Chem.*, **68**, 759 (1926).

(7) Folin, O., "The Determination of Sugar in Blood and Normal Urine (Revised)," *Ibid.*, **67**, 357 (1926).

(8) Benedict, S. R., "The Determination of Blood Sugar," *Ibid.*, **76**, 457 (1928).

(9) Folin, O., "Two Revised Copper Methods for Blood Sugar Determination," *Ibid.*, **82**, 83 (1929).

(10) SUMNER, J. B., "A More Specific Reagent for the Determination of Sugar in Urine," *Ibid.*, **65**, 393 (1925).

(11) KINGSBURY, F. B., "Note on the Effect of Glucose on the Condensation of Formaldehyde. I. The Determination of Urinary Sugar by This Principle," *Ibid.*, **75**, 241 (1927).

(12) ROTHBERG, V. E., and EVANS, F. A., "Studies with the Folin and Wu Blood Sugar Determination," *Ibid.*, **58**, 435 (1923).

(13) FOLIN, O., and DENNIS, W., "The Determination of Lactose in Milk," *Ibid.* **33**, 521 (1918).

(14) BIERMAN, H. R., and DOAN, F. J., "A Colorimetric Picric Acid Method for Determining Lactose," *J. Dairy Science*, **7**, 381 (1924).

(15) MUNSON and WALKER, General Method, *A.O.A.C. Methods of Analysis*, p. 571, 6th Ed. (1945).

(16) KLEMME, DOROTHEA, and POE, C. F., "A Study of Methods for the Determination of Reducing Sugars in Bacterial Cultures," *J. Bact.*, **32**, 1 (1936).

(17) BROWNE, C. A., and ZERBAN, F. W. "Physical and Chemical Methods of Sugar Analysis," pp. 321, 882, 3rd. Ed. John Wiley and Sons, New York. 1941.

CRYSTALLINE CHARACTERISTICS OF FOUR CAROTENE ISOMERS

FRANCIS T. JONES and E. M. BICKOFF (Western Regional Research Laboratory,[1] Albany, Calif.)

This paper consists of a partial description of the crystallographic and optical properties of the four carotene isomers whose absorption spectra have been reported by E. M. Bickoff, L. M. White, A. Bevenue, and K. T. Williams.[2] This description of these carotinoids is given at this time because it seems unlikely that any more of the unstable carotene isomers will be available in the near future. It is hoped that this partial description will be found useful by others working on these materials.

The isomers were prepared from a commercial carotene product by chromatographic adsorption methods given in detail by the authors cited above.[2] After separation of alpha-carotene, the beta-carotene was isomerized with iodine and then fractionated. The crystals were grown by addition of methanol to a benzene solution of each isomer in a centrifuge tube and allowing the mixture to stand in a refrigerator. After crystals had grown, the tube was centrifuged and the supernatant liquid was decanted. Portions of the solid suspended in a little of the saturated solution were placed on a microscope slide, covered with a cover glass and then examined both before and after the solvent had evaporated. Attempts to determine refractive indices failed, because the crystals were soluble in the index liquids (methylene iodide solutions) except for a potassium mercuric iodide solution whose index was too low in most cases.

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[1] Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

[2] Isolation and Spectrophotometric Characterization of Four Carotene Isomers. E. M. Bickoff, L. M. White, A. Bevenue, and K. T. Williams. *This Journal*, **31**, 633 (1948).

The indices are in the neighborhood of 1.7 for sodium light as noted for each isomer below. Pleochroism is strong in most cases although it is not readily observed on thick crystals and varies somewhat with the orientation. Very thin crystals may be pale yellow, thick crystals will be opaque.

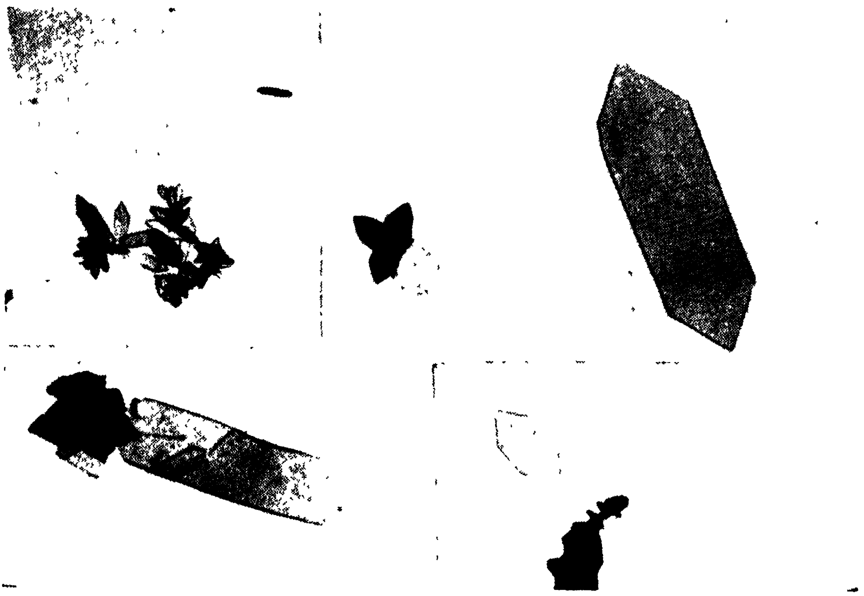

FIG. 1. All-trans-beta-carotene. 100X.

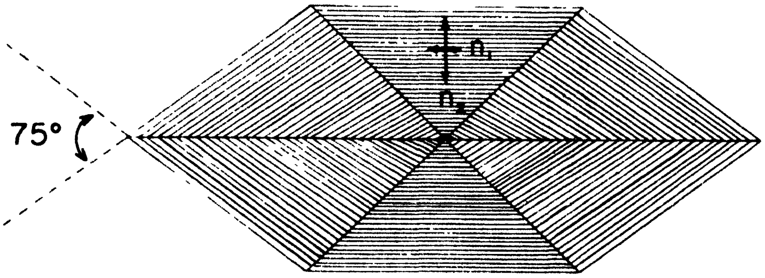

FIG. 2. —All-trans-beta-carotene showing zoning.

Samples of commercial carotene have shown plates of the all-trans-beta carotene type which, if unoxidized, dissolve completely when a drop of benzene is allowed to run under the cover glass during microscopical observation, but if the crystals have suffered partial oxidation an insoluble colorless residue will remain as a "pseudomorph" of the original crystal. The amount of this residue will increase with increase in oxidation. The crystals separating at the edge of the cover glass as the benzene evaporates are usually platy but may include some patches made up of fine grains in dendritic patterns as the drop dries. Birefringence and pleochroism are strong.

All-trans-beta-carotene.—Photomicrographs of crystals of this isomer are shown in Figure 1. The habit is platy with a tendency to cluster. The crystals are often thin enough to show thin film interference colors. The plates are six sided in outline and from two to four times as long as they are wide. The termination is usually imperfect, often showing a shoulder at the base of an acute termination, but in some cases the end angle is obtuse. Those crystals which seemed most nearly perfect had a terminal angle of about 75°. Close inspection reveals that these plates are often composed of segments as shown in Figure 2. These segments may be due to twinning. Under polarized light between crossed nicols these segments do not all extinguish together but opposite pairs extinguish together. The pair of segments at the sides of the plate are extinguished when the plane of vibration is parallel to the length of the crystal but a slight rotation is necessary to bring either of the other pairs of segments to extinction. The slow ray (n_1) is lengthwise of the crystal and the pleochroism shows the darkest orange color for lengthwise vibrations, with a lighter yellowish orange for crosswise vibrations (n_2).

FIG. 3. All-trans-alpha-carotene. 100×.

The side segments show a higher refractive index than their adjacent segments as judged by the movement of the Becke line at the boundary

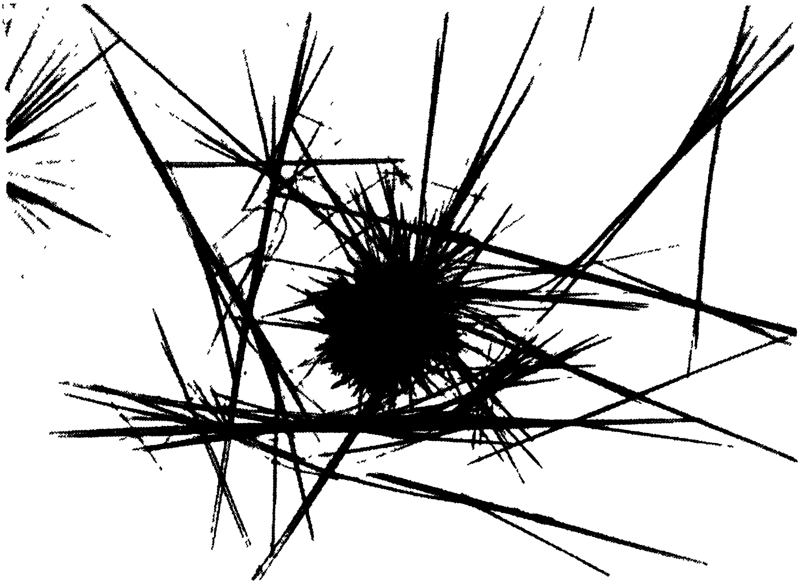

FIG. 4. Neo-beta-carotene-U. 100X.

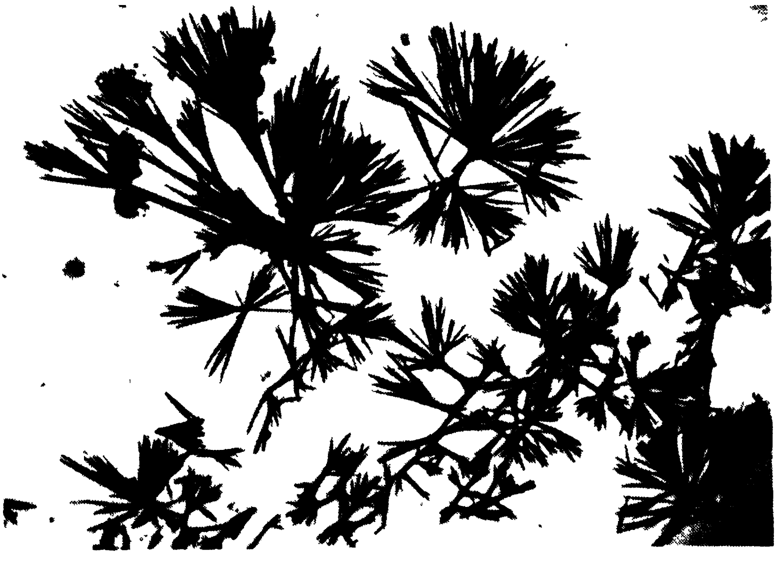

FIG. 5. - Neo-beta-carotene-U. 100X. Grown on microscope slide.

within the crystal. The difference is smaller for crosswise than for lengthwise vibrations.

These crystals dissolved in index liquids composed chiefly of methylene iodide. They appeared to be stable in an aqueous solution of potassium mercuric iodide (Sonstadt's or Thoulet's soln), n = 1.68, but their refractive indices were considerably higher than this value.

All-trans-alpha-carotene.—Crystals of this isomer are shown in Figure 3. These crystals are very similar to those of all-trans-beta-carotene. Thin film interference colors are responsible for the banding seen in many of these crystals. The terminations are more nearly perfect on these crystals as compared to the all-trans-beta, and the zoning, when present appears to be due to the superimposition of several crystals in such a way as to give greater thicknesses of some zones than others. The description of unsolvated carotene crystals given by F. G. Kohl[3] appears to be most nearly in agreement with the properties of the alpha and beta isomers reported here.

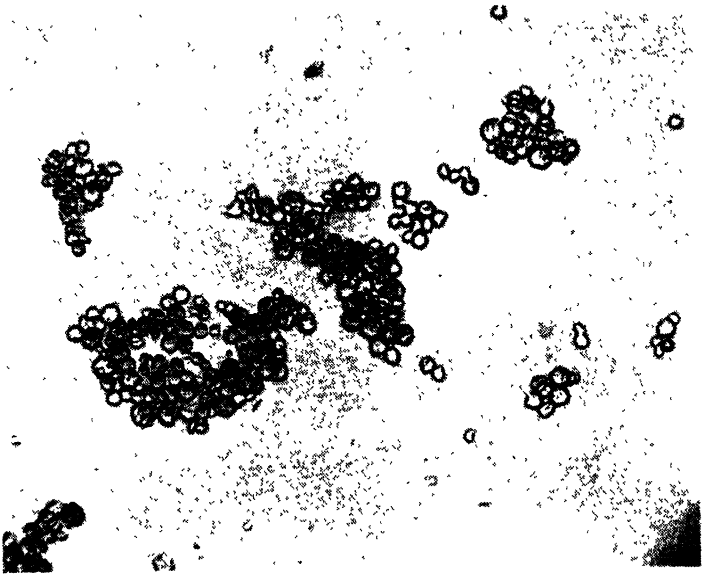

FIG. 6.—Neo-beta-carotene-B. 100×.

Neo-beta-carotene-U.—Crystals of this isomer are shown in Figures 4 and 5. These crystals are conspicuously different from the all-trans-alpha or all-trans-beta isomers. They habitually form tapering needles of narrow blades which tend to cluster into loose sheaves and tufts as seen in Figure 5. The crystals in Figure 5 were grown on a slide by addition of methanol to a benzene solution under a cover glass. In polarized light the extinction is parallel. The slow ray is crosswise, corresponding to the strongest absorption which gives a yellowish brown color. For lengthwise vibrations the color is light yellow. Both refractive indices are less than the 1.68

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[3] F. G Kohl, Untersuchungen über das Carotin, Verlag von Gebruder Borntraeger, 1902, Leipzig.

value of the Sonstadt's solution used, contrasting in this property with the all-trans-alpha and all-trans-beta isomers. These characteristics agree with those reported by A. Polgar and L. Zechmeister.[4]

Neo-beta-carotene-B.—Crystals of this isomer are shown in Figure 6. These crystals grow only in spherulitic aggregates whose crystalline nature is revealed under polarized light. The rounded masses are dark red at first but become almost colorless as a result of oxidation after standing in air on the slide for a day or two. Immersion in Sonstadt's solution greatly retards this oxidation. Between crossed nicols the rounded masses show a spherulitic cross in which the slow ray is tangential as determined with a quartz wedge in sodium light. Both refractive indices are greater than 1.68. Many of the rounded masses are partially fused together, sometimes in chains, and some of them are isotropic, indicating that they are still liquid. It is possible that the carotene separates first in oily globules which partially run together before or during crystallization. After oxidation the colorless masses resulting are isotropic.

DETECTION AND DETERMINATION OF COCAINE IN THE PRESENCE OF PROCAINE AND VARIOUS OTHER SUBSTANCES

By James L. Young (Alcohol Tax Unit, Bureau of Internal Revenue, Washington, D. C.)

The detection of cocaine, in the presence of procaine, offers one of the most difficult problems confronting the chemist engaged in analysis of samples involved in litigation under the Harrison Narcotic Law.

These two substances are not only soluble in the same solvents, but form crystalline complexes with the "alkaloid reagents," which are so much alike as to defy positive identification.

Consideration of these compounds from the standpoint of decomposition and the subsequent identification of the by-products formed, led to the present approach to the problem.

Cocaine, being the methyl ester of benzoyl ecgonine, yields methanol on hydrolysis with an alkali. Procaine, on the other hand, not being a methyl ester, does not.

The methanol resulting from the hydrolysis of cocaine is determined by Beyer's[1] modification of the Georgia-Morales[2] test, since this method produces a color of such stability that comparison with test standards is practicable.

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[4] A. Polgar and L. Zechmeister, *J. Am. Chem. Soc.*, 64, 1856 (1942).
[1] *This Journal*, 22, 151 (1939).
[2] *J. Ind. Eng. Chem.*, 18, 1812–3 (1926).

EXPERIMENTAL

Mixtures of cocaine with procaine and varying proportions of other synthetic drugs, as well as the opium alkaloids and such diluents as lactose, starch, and cane sugar, were hydrolyzed and the resulting methanol determined colorimetrically. None of the substances mixed with the cocaine gave positive tests alone, nor did they influence the test in mixtures. Only compounds which yield methanol on mild hydrolysis should present any problem of interference. The sensitivity of the test was ascertained to be approximately 2 mg of cocaine hydrochloride.

METHOD (QUALITATIVE)

REAGENTS

Approximately 2% aqueous solution of NaOH.

Approximately 24% Ethyl Alcohol (by volume).

Potassium permanganate soln:—Dissolve 3 g $KMNO_4$ and 15 ml of sirupy phosphoric acid (85%) in 100 ml of distilled water.

Oxalic-sulphuric acid soln:—Dissolve 5 g of $H_2C_2O_4$ in 100 ml of $H_2SO_4(1+1)$.

Modified Schiff's reagent (Fuchsin-Sulfurous Acid T.S., U.S.P. XIII):—Dissolve 0.2 g of Kahlbaum's rosanaline HCl in ca 120 ml of hot water. Cool, and add 2 g of Na_2SO_3 previously dissolved in 20 ml of water. Add 2 ml of concd. HCl, dilute the soln to 200 ml, and place in the refrigerator for at least 24 hours before using for quantitative determinations. Store in a cool place.

DETERMINATION

Introduce 25–50 mg of the alcohol-free sample into a round bottom long neck distilling flask of ca 25 ml capacity. Add 2.0 ml of NaOH followed by 5 ml of water.

Connect the flask to a small condenser and carefully distill until 2 ml of distillate is collected in a 25 or 50 ml glass stoppered cylinder. (Ground glass joints should be used on all connections.) To the 2 ml of distillate add 0.25 ml of 24% ethyl alcohol and 2.75 ml of water. Add 2 ml of $KMNO_4$ reagent and allow to stand with occasional agitation during 10 minutes. Now add 2 ml of oxalic acid reagent followed by 5 ml of Schiff's reagent. Invert the cylinder three times and tightly stopper at once. Examine the cylinder after one and two hours viewing thru the long axis. A blue or violet color indicates the presence of cocaine in the sample. The intensity of color is a function of the amount of cocaine present.

METHOD (QUANTITATIVE)

Standard cocaine solution:—Accurately weigh 50 mg of pure cocaine hydrochloride and dissolve in 10 ml of distilled water; 0.1 ml of this solution equals 0.5 mg of cocaine hydrochloride.

DETERMINATION

Weigh 25 mg of the sample (freed from alcohol if necessary), introduce into a 25 ml round bottom flask and follow the Qualitative Method with the following modifications. Collect the 2 ml distillate in a 6-inch Nessler tube having a 2 ml marking. Introduce 0.4 ml, 0.6 ml, and 1.0 ml portions, respectively, of the standard cocaine soln into separate distilling flasks and proceed as under Qualitative Method, collecting the 2 ml distillates in 6-inch Nessler tubes. After standing two hours, make trial comparison of the color developed by the sample and by the standards.

(The oxidation of the sample and the standards with the permanganate reagent should be started at the same time.) If the color developed in the sample exceeds that of 1.0 ml standard, a smaller sample must be employed, which can be accomplished by dissolving a suitable weight of the sample in water or 0.02 *N* HCl and using an aliquot for analysis.

For accurate work the final comparison should be made with a series of standards in 0.1 ml increments. If suitable photometric equipment is available, a permanent standard curve may be prepared from extinction readings at 560 millimicrons.

SUMMARY

A method is presented for the detection and determination of cocaine alone, or in mixtures. The alkaloid is hydrolyzed and the methanol resulting from the hydrolysis is determined colorimetrically. The method is accurate, rapid, and requires a minimum of manipulation and apparatus

MOLD MYCELIA COUNT METHOD FOR CRANBERRY SAUCE

By William V. Eisenberg and Albert H. Tillson (U. S. Food and and Drug Administration, Federal Security Agency, Washington, D. C.)

It has been recognized for a number of years that the mold mycelia count method as used for tomato products is also applicable to cranberry products for the purpose of detecting the use of rotten fruit. The method as used by many laboratories has consisted of making a mold count directly on a portion of the gelled sauce.

The writers recently conducted a survey for the purpose of testing this method and establishing the relationship between the mold mycelia count and the condition of the fruit being used. At the outset of this survey it was observed that the direct method of counting was not entirely suitable, since the microscopic field was often too dense to permit clear vision and prompt detection of the mold mycelia in the cellular mass. This condition was observed in making counts at the factory of both the pulp and the finished sauce. The use of a pectin solution for dilution purposes, such as is used in making mold counts of many other fruit products offering the same diffiulty, seemed highly desirable. (See Figs. 1 and 2.) Accordingly, the following method was devised for use in making counts of both the strained and whole berry sauces.

METHOD

(a) *Strained Sauce*

Immerse the unopened can of sauce in a boiling water bath for 30–45 minutes, in order to facilitate breaking the gel. Remove can from bath and open carefully to avoid loss of sauce thru sudden release of pressure. Empty contents of can into a suitable sized beaker (1 liter beaker for #2 can). Stir the sauce in order to break the gel. A slow-speed electric mixer (350–450 r.p.m.) may be used for this purpose.

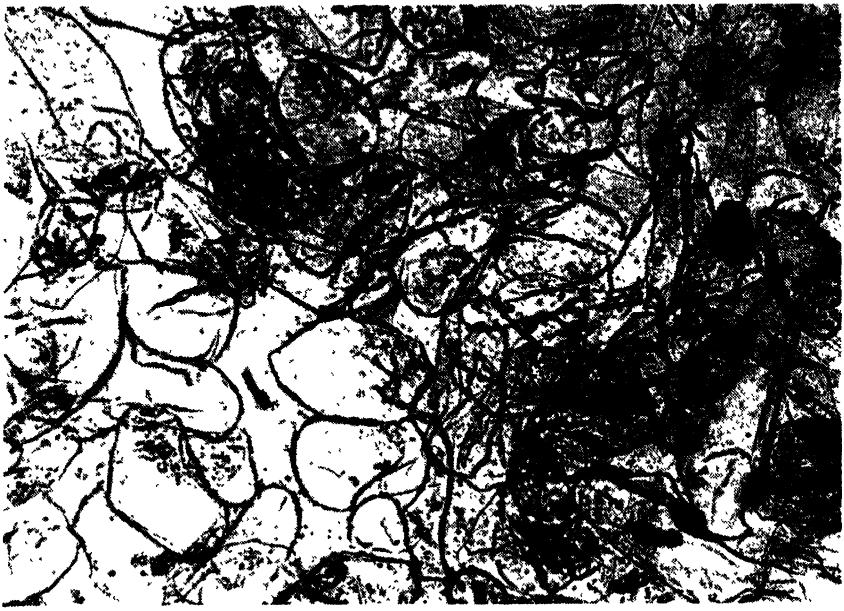

FIG. 1.—Undiluted strained cranberry sauce on mold counting chamber. ×100.

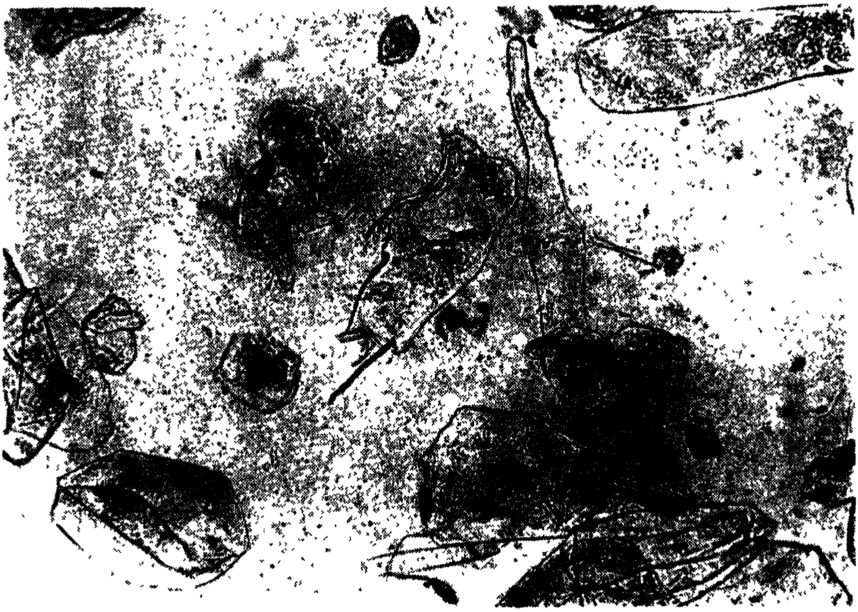

FIG. 2.—Diluted strained cranberry sauce (1+1) on mold counting chamber. ×100. Note mold mycelia along the cell wall and in interior of cell at (a).

Mix thoroly 50 g of the stirred sauce with 50 g of a 3% pectin soln.[1] Make a mold count of this mixture using official mold count method as directed in *Methods of Analysis*, 6th Ed., Sec. 42.57.

(*b*) *Whole Sauce* (*Seeds and Skins Included*)

Pulp contents of container (if considerably greater than 1 lb, such as #10 can, remove well-mixed aliquot of 1 lb) thru cyclone with screen openings ca 0.027" in diam. This will remove skins and seeds and prepare a homogeneous pulp for mold counting. Mix 50 g of pulp with 50 g of 3% pectin soln. Make mold count of this mixture as directed in *Methods of Analysis*, 6th Ed., Sec. 42.57.

Manufacturing plants using this method for control purposes can take samples of the strained pulp or the finished strained sauce from the production line and make their dilutions directly, thus foregoing the need for breaking the gel as directed in the method for strained sauce. The writers have found, however, that control is best exercised on the sorting belt in in order to eliminate rotten fruit.

Collaborative work was undertaken in order to test the methods. Diluted samples were prepared for mold counting and these were rotated among the analysts, each preparing his own slides as directed in the method. These analyses were made of sauces examined during the field survey at various plants and later in the laboratory. The material used consisted of cranberry products sampled in the Cape Cod and the New Jersey areas.

The comparative results are tabulated below:

Comparison of mold count results on same sauces by three different analysts

SAMPLE NO.	MOLD COUNTS			
	WVE	AHT	FRS*	AVERAGE
		Strained Sauce		
1	46	52	—	49.0
2	38	40	—	39.0
3	68	72	—	70.0
4	50	48	—	49.0
5	26	32	—	29.0
6	44	42	36	40.7
7	34	34	40	36.0
8	76	78	76	76.7
9	76	80	74	76.7
		Whole Sauce		
10	10	14	—	12.0
11	4	6	2	4.0
12	14	16	6	12.0

* FRS participated only in the mold counts made in the laboratory.

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[1] See *Methods of Analysis*, 6th Ed., Sec. 42.2(b) for preparation of the pectin soln.

These comparative results are influenced by two factors, first, the sampling variation in removing a portion for preparing the counting chamber and the distribution of the mold among the microscopic fields in the counting chamber and second, the personal variation among analysts. The analytical results show good agreement among the analysts. The variation can be accounted for to a large extent by the first factor mentioned above, namely, sampling variation.

The results of this study demonstrate conclusively the validity of this method for the quantitative determination of mold mycelia in cranberry sauce. Concordant results can be obtained by qualified analysts trained in the use of this method.

TESTS ON THE EFFICIENCY OF VARIOUS FILTH RECOVERY PROCEDURES

II. INSECT FRAGMENTS AND RODENT HAIRS FROM FLOUR*

By KENTON L. HARRIS and LILA F. KNUDSEN (United States Food and Drug Administration, Federal Security Agency, Washington 25, D. C)

In August 1940 the Food and Drug Administration issued a mimeographed "Method for the Recovery of Filth from Flour, Revised." This method evolved out of the work of several Administration analysts: W. S. Greene had used saturated salt solution to float rodent hairs and insect fragments from flour. Analysts at the New Orleans Station had used the same principle but had modified the procedure. The Wildman trap flask, employed at that time for tomato products, was used in a saturated-salt-gasoline method described by the Cincinnati Station and the Microanalytical Division. The latter method was rewritten by W. H. King (New Orleans Station), tested and modified by K. L. Harris and W. G. Helsel (Microanalytical Division), and issued in a mimeographed form September 1938. The "Manual of Microanalytical Methods," compiled in 1943 for use by analysts of the Food and Drug Administration, contained this same method, condensed in wording. In addition a second extraction, by the following technique, was called for after the first trapping off in the first trap flask:

> "Add 10 to 20 ml . . . gasoline . . . to the trap flask; stir in vigorously; add ca 10 ml saturated NaCl soln; allow to stand 10 to 20 minutes with intermittent stirring of the bottom layer; trap off and filter."

J. F. Nicholson and S. M. Walden (1) (Baltimore Station), in March 1944, reported a pronounced increase in the insect fragment recovery from six samples of contaminated flour when the flour was first digested with an aqueous extract from pancreatin.[1] They concluded that the pancreatin released insect fragments that would otherwise be held down in the flask by the protein; and also because the sticking of insect fragments to protein is unpredictable, that the use of a pancreatic digestion would tend to produce more uniform results. A method based upon their work was added to the U. S. Food and Drug Administration "Manual of Microanalytical Methods."

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* Part I, by Harris and O'Brien, appeared in *This Journal*, 29, 84 (1946).

Working independently, analysts of the Atlanta Station and the Microanalytical Division used saturated salt solution in the trap flask in place of the water recommended in the above method. They felt that the increased specific gravity of the salt solution would aid in the flotation of insect parts.

The following method given in *Methods of Analysis* of the Association of Official Agricultural Chemists, Sec. **42.30** (amended), is based upon the results of all these investigations:

Insects, insect fragments, and rodent hairs. Weigh 50 g of flour into 250 ml beaker. Add ca 60 ml pancreatin soln (prepared as a filtered aqueous extract of 5 g pancreatin per 100 ml H_2O) and stir into smooth paste. Add ca 40 ml of the pancreatin soln (100 ml total) and mix. (Adjust to *p*H 7–8 with ca saturated Na_3PO_4 soln.) Allow to stand ca 15 min, and if necessary readjust to *p*H 7–8. Maintain at 40° for not less than 3 hours. Transfer digested material to liter Wildman trap flask. Add 20 ml of gasoline and mix thoroly. Allow mixture to stand 5 min., fill with saturated NaCl soln, and after 30 min. trap off into 250 ml beaker. Add ca 10 ml gasoline to the material in the trap flask, stir the gasoline into the mixture and after ca 5 min., trap off into the same beaker. Transfer contents of beaker to trap flask and fill with saturated NaCl soln. Stir and after ca 30 min. trap off into beaker[2] and filter thru rapid filter paper, using suction. Examine microscopically.

Aside from the recovery work by Nicholson and Walden the development of the present method for the detection of insect fragments and rodent hairs from flour has been accompanied by no comparative recovery tests. Changes that appeared reasonable, or which increased the ease of handling, or which permitted more rapid microscopic examination by incorporating less flour residue in the floating layer, were simply accepted and used by the analysts doing the great bulk of this type of work. The use of a second trapping has become well established in this laboratory although, aside from the work by Harris and O'Brien (1946) (2), there has been no published data. These analysts concluded: "The second extractions in a Wildman trap flask yield appreciable amounts of filth. In general, where recoveries are low on the first extraction they are higher on the second. Therefore, the use of two extractions on all samples will give more uniform results than will one extraction." Eisenberg (1947) (3) working with spices found that, when appreciable amounts of filth were found in the first extraction, additional fragments were found in the second, as shown in Table 1.

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[1] The first reference to the use of the enzyme pancreatin to release contaminants from foods is in a mimeographed publication "Examination of Bakery Products for Filth," by Harry Shuman, Philadelphia Station, U. S. Food and Drug Administration, February 19, 1941. Although pancreatin has since been widely used in the analysis of food for filth, there has been no previous mention in the published literature of the technique as used by Shuman.

[2] Under "Changes in Methods of Analysis," *This Journal*, 30, 90 (1947) the following addition has been made to sec. 42.4(a) as a sixth paragraph: "When the trapped-off material contains an appreciable amount of starch material, add sufficient conc. HCl to make 1–2% HCl, boil, and filter while hot." To preserve the uniformity in the procedures being tested, all trapped-off portions reported in this paper were treated in this manner.

TABLE 1.—*Insect fragments and rodent hairs recovered by the first and second extraction of spices**

(Insect parts and rodent hairs from different samples)

INSECT FRAGMENTS		RODENT HAIRS	
1ST EXTRACTION	2D EXTRACTION	1ST EXTRACTION	2D EXTRACTION
17	1	1	0
0	1	1	0
3	0	1	0
3	0	1	0
24	1	0	4
203	33	8	5
19	1	0	2
4	1	1	0
17	3	1	0
5	0	5	1
71	15	1	0
5	1		
23	4		
19	5		
1	0		
38	3		
45	4		
1	0		
3	0		
42	2		

* Eisenberg, 1947.

Helsel and Harris in previously unpublished data (see Table 2) showed significant amounts of filth recovered from corn meal in the second trapping.

These results, plus the common observation in this laboratory of insect fragments floating in the oily layer of the so-called second extraction, leave no doubt but that the second trapping off recovers an appreciable number of insect parts and rodent hairs when they are present in the material being examined.

EXPERIMENTAL

In order to evaluate the other specific details leading up to and tentatively incorporated in method **42.30**, a series of tests were made following the plan started in Part I, *loc cit.*, p. 84, in which an artifically contaminated lot of flour was used as the basic contributor of the filth to be extracted. A 100-pound batch of patent flour was mixed with a 20-pound lot of flour heavily contaminated with insect fragments, finely ground rodent excreta, and rodent hairs. A preliminary examination of this resulting flour indicated that the insect fragment and rodent hair counts were so high that it would be impractical to count the filth extracted from it.

TABLE 2.—*Rodent hairs and insect fragments recovered by the first and second extractions from corn meal**

RODENT HAIRS		INSECT FRAGMENTS	
1ST EXTRACTION	2D EXTRACTION	1ST EXTRACTION	2D EXTRACTION
38	1	281	30
10	0	502	27
23	5	196	56
5	1	68	26
6	1	55	26
5	0	382	79
5	5	350	111
10	5	274	67
18	6	342	67

* W. G. Helsel and K L Harris, unpublished.

For this reason, 7 grams of this dirty flour were mixed with 43 grams of a clean, commercial, general purpose, patent household flour to make the 50 gram portions of flour used in tests of various filth extraction methods.

A. Comparison of various techniques which contributed to the development of the 1945 A.O.A.C. method: section 43.20

Ten 50-gram portions of flour prepared as described above were tested by each of eight variations of the basic method, in such a manner that data was obtained on filth recoveries by the above-mentioned method by 4 variable procedures (1) trap flask size, (2) extraction of medium, (3) pretreatment, with chloroform as given in section 42.29 of *Methods of Analysis*, (4) digestion with pancreatin. The analytical results are given in Table 3 and shown graphically in Figure 1.

1. Trap flask size.—Series 1 and 2, 3 and 4, and 6 and 7 were similar procedures varying within the pairs only by the trap flask size. By means of analysis of variance, a comparison of the recoveries obtained shows that there was no significant difference introduced by flask size. Previous experience has demonstrated that, when handling lower grades of flour, less flour debris will be deposited on the filter paper if a 2-liter trap rather than

TABLE 3.—*Rodent hair and insect fragment recoveries from flour*

SERIES	TYPE FILTH	EXTRACTION PROCEDURE				RESULTS PER 50 GRAM SUBDIVISIONS													
		CHLOROFORM	DIGESTION	FLOT. MED.	TRAP SIZE	INDIVIDUAL SUBDIVISION COUNTS										SUMMARY			
		(VAR. 3)	(VAR. 4)	(VAR. 2)	(VAR. 1)	1	2	3	4	5	6	7	8	9	10	HIGH	AVE.	LOW	S.E. AVE.
1	Hairs Insects	No	Pancr.	Water	1	12 194	20 162	13 172	13 201	19 229	13 190	9 163	21 156	19 171	13 154	21 229	15 179	9 154	1.3 7.6
2	Hairs Insects	No	Pancr.	Water	2	20 199	16 169	20 183	17 214	19 174	10 222	15 147	20 230	25 175	18 175	25 230	18 189	10 147	1.2 8.4
	Hairs Insects	No	Pancr.	Salt	2	9 153	9 173	12 171	13 186	16 177	6 143	10 184	17 196	23 185	11 147	23 196	12 172	6 143	1.6 5.7
4*	Hairs Insects	No	Pancr.	Salt	1	11 144	13 174	17 173	9 182	11 163	15 173	16 161	14 186	9 149	16 146	17 186	13 165	9 144	0.9 4.7
5	Hairs Insects	Yes	None	Water	2	5 225	6 224	7 298	8 217	3 241	0 18	0 20	0 0	0 2	0 16	8 298	3 126	0 0	1.0 39.0
5A	Hairs Insects	Yes	None	Salt	2	0 78	1 66	0 35	0 72	0 51	0 97	0 64				1 97	0 66	0 35	0.2 7.4
6	Hairs Insects	No	None	Salt	2	18 159	8 201	19 184	21 219	15 172	15 210	19 181	15 171	14 190	13 160	21 219	16 185	8 159	1.2 6.4
7	Hairs Insects	No	None	Salt	1	6 155	21 188	11 188	13 199	14 162	13 162	7 153	18 184	14 146	7 158	21 199	12 170	6 146	1.5 5.8
8	Hairs Insects	No	Dil. HCl Paste	Acidified Water	1	13 127	17 133	15 121	16 112	11 116	11 112	15 120	13 108	11 183	13 176	17 183	14 130	11 112	0.7 8.5
9	Hairs Insects	No	Dil. HCl-Slurry	Acidified Water	2	15 191	25 162	11 187	9 203	12 240	18 240	13 168	19 264	18 242	19 243	25 264	16 214	9 162	1.5 11.4
10	Hairs Insects	No	HCl-Acetone	Acid. Acetone-wat.	Separate Funnel	15 257	16 278	17 203	15 246	15 286	4 72	11 262	15 249	11 153	20 259	20 286	14 226	4 72	1.4 21.1
11	Hairs Insects	Directly through #100 Screen				4 58	8 63	5 50	7 56	11 48						11 63	7 55	4 48	

s.e. Ave. = Standard error of the average.
* *Methods of Analysis*, 6th Ed. sec. 42.30.

a 1-liter trap is used. Therefore, since no loss of filth is involved, the use of a 2-liter flask is recommended.

2. Extraction medium.—As tested by an analysis of variance comparing series 1 and 4 and 2 with 3, there is a significantly higher recovery of both insect fragments and rodent hair fragments when water is used in place of the saturated salt solution. This finding is contrary to that expected by analysts who thought that, because of its higher specific gravity, saturated aqueous sodium chloride would float more filth into the oily layer; but agrees with the reasoning that it is easier to wet (with oil) the filth in liquids of lower surface tension and so enable them to be carried by the oil into the floating layer. Helsel and Harris, in devising a method for the isolation of filth from corn meal, found that a higher insect fragment recovery was obtained by trapping off in 60% ethanol than in 40% ethanol. In spite of the lower specific gravity an unimpaired recovery was obtained in alcohol water solutions as compared with water alone. It therefore seems reasonable to conclude that insect fragments and rodent hairs are more readily wet by oil when in solutions of low surface tension and thus are more completely extracted into the oily floating layer.

3. Pretreatment with chloroform.—Pretreatment according to **42.29** is as follows:

> Weigh 50 g of flour into 250 ml beaker. Add $CHCl_3$ to within ca 1 cm of top of beaker, mix thoroly, and allow to settle at least 30 min. Several times during this period, stir layer that rises to top. Decant soln and floating flour, being careful not to disturb heavy residue in bottom of beaker. Before decanting, take care that floating layer has not become so compact as to render this operation difficult. Wash down sides of beaker with stream of $CHCl_3$ from wash bottle. If so much material remains that it would interfere with subsequent microscopic examination, repeat decanting after adding more $CHCl_3$ until very little flour is left in beaker. Take care not to decant any heavy filth that may be present. Wash heavy residue remaining in beaker with stream of $CHCl_3$ onto ruled filter paper in Hirsch funnel. Transfer filter paper to Petri dish and examine microscopically.

The choice is then optional whether the subsequent analysis under **42.30** for insect fragments and rodent hairs should be carried out on the same or a new aliquot. Scattered and unreported observations in this laboratory indicated that the flotation in chloroform might make subsequent extractions for light filth erratic. In order to test this, portions of the flour were treated as in **42.29** except that all of the material was decanted into the funnel, dried, and then extracted for insect fragments and rodent hairs.

The results of these tests show that the reliability of the method is markedly diminished when the same portion that is to be extracted for light filth has been previously treated by the chloroform sedimentation procedure. The flours in series 5 (Table 3), which previously had been treated with chloroform were tested in two groups each of five 50-gram portions. Subdivisions 1 to 5 all showed higher insect fragment recoveries and lower rodent hair fragment recoveries than other methods. Subdivisions 6 to 10 in series 5 all showed low insect fragment recoveries, and no hairs, whatever were obtained. The same analyst tested both groups, and the same apparatus was used. Moreover, at the same time as each of these groups of five were being tested, another group was being tested by one of the other procedures without resulting in such marked divergence. In addition to these ten portions, seven other tests had been run in conjunction with some preliminary work. The results of these seven analyses are given in Table 3, series 5 A. Although 5 and 5A differ in the flotation medium used, the recoveries are definitely low.

J. F. Nicholson, U. S. Food and Drug Administration, states (personal communication) that in routine testing, he has encountered unexpectedly low results in chloroform-treated samples which he had every reason to believe were contaminated. In instances where he made a repeat examination on separate protions untreated with chloroform, the results were higher.

It seemed possible that the low recoveries in 5A were due to the removal by chloroform of the oleophilic coating naturally present on rodent hairs and insect cuticle. However, insect fragments and rodent hairs which had been extracted for seven hours in running chloroform were subsequently completely recovered from a gasoline-water mixture (no food present). Furthermore, previously unpublished data by Helsel and Harris, as given in Table 4, show higher insect fragment and rodent hair recoveries in corn meal after soaking in chloroform. If the poor recovery in flour is due to the extraction of oils from hairs and insects, then these results on corn meal do not necessarily refute this argument, since there is so much oil in whole corn meal that the relatively crude extraction would be incomplete, although the *improved* recovery still remains unexplained.

TABLE 4.—*Rodent hairs and insect fragments from duplicate 50-gram samples of whole corn meal, with and without previous* $CHCl_3$ *sedimentation**

No $CHCl_3$	Hairs	19	4	5
	Insect fragments	142	139	100
With $CHCl_3$	Hairs	30	5	8
	Insect fragments	196	234	137

* W. G. Helsel and K. L. Harris, unpublished, 1939.

4. Pancreatic digestion.—In comparing a flotation preceded by pancreatin digestion with the flotation of undigested flour, the undigested flour was made into a paste in the extraction medium, as would be done with pancreatin, before diluting it and extracting in the trap flask. A comparison of the recoveries obtained in series 3 and 6, and 4 and 7 shows no statistically significant difference in either insect fragment or rodent hair recoveries on the flour used here.

However, Nicholson and Walden (1944) observed (1) that in the handling of routine laboratory samples they had less trouble with the formation of emulsions when pancreatin was used before the trapping operation; and that on flour from which they recovered less filth than might be expected from the previous history of the sample, they were able to approach more closely the expected results by using pancreatin on the repeat analysis. After they started to use a pancreatin-digestion procedure, they noted a decrease in the number of samples which were difficult to handle. Moreover, on the six comparative samples tested by direct flotation and by flotation after pancreatin digestion they found an increased recovery on the pancreatin treated portion.

These tests, reported in Table 5, were made on five samples of soft wheat general purpose flour and one sample of soft wheat second clears (low grade) flour, each from a different mill, and each selected for re-testing with pancreatin because the original results were unexpectedly low. Although these data were obtained by using two different extraction fluids (in addition to the pancreatin variable) the results are significant.

TABLE 5.—*Insect fragments from six different 50-gram samples of flour by two procedures*

Nondigested, sat. salt[1]	116[2]	26	12	94	112	41
Pancr. digested, water	868	127	116	352	134	77

[1] The basic difference was the use or non-use of pancreatin.
[2] Second clears flour; others were general purpose.

In discussing these results and their influence upon recommendations to be made as to the best method to use for flour, it is important to note that certain flours are more difficult to handle than others. A high patent flour, such as was used in the experimental work reported in this paper, contains less bran and protein than the lower patents and low grade flour reported by Nicholson and Walden. It is the author's (H.) experience that the lower grades are the ones which give more flour and bran debris floating into the oily layer and so make filter papers which are more difficult to read. It is his experience also that the use of pancreatin before extracting filth will give filth papers bearing less extraneous cereal debris and so permit more rapid filtration and more rapid microscopic examination, without the need of probing thru cereal tissues that might confuse less experienced analysts.

Thus the use of pancreatin in no way lowers the recovery; it may increase the recovery from some flours; and it permits the more rapid handling of routine samples.

B. Digestion in dilute HCl

The addition of a pancreatic digestion procedure to the method increases the analytical time, and some thought has been given to a procedure that will rapidly break down some of the proteins and starch. Boiling in dilute hydrochloric acid will accomplish this and has been suggested for several cereal products.[3] Two such procedures are discussed in this section.

1. Digestion in 200 ml 2% HCl:

To 50 g flour in a beaker add sufficient 2% HCl to form a thick paste. Stir until smooth. Add 2% HCl to make total of 200 ml. With constant stirring bring to boil. Cool. Transfer to 1-liter trap flask and extract with gasoline and H_2O in the usual manner.

The results by this method are reported in Table 3, series 8. The hair recovery was good, with the least variable results. The insect fragment count is slightly lower than with the pancreatin procedures.

2. Digestion in 400 ml 2% HCL.

The American Association of Cereal Chemists (Mimeo. 1946) (4) reported a similar procedure but with a larger volume of dilute hydrochloric acid, and with mineral oil added to the boiling mixture. In view of this work, and since the smaller volume of flour had a tendency to adhere to the bottom of the beaker and scorch, the following method was used:

To 50 g flour in a beaker add water and stir into a thin smooth paste. Add H_2O to make 400 ml total H_2O added. Add conc. HCl to make total HCl of water 2%. With intermittent stirring bring to a boil and boil 20 min. Transfer to 2 liter trap flask and trap off with gasoline and water in the usual manner.

This hydrochloric acid treatment with a greater volume of solution' longer boiling time, and larger flask size gave a high recovery of insect fragments (see Table 3, series 9). In fact, a comparison of these results with any of the pancreatic digestion recoveries shows the hydrochloric acid recoveries to be higher. The rodent hair recovery is not significantly different from all of the pancreatin digested series.

C. Extraction in separatory funnel

Walker and Dalby (1947) (5) report on hydrochloric acid-acetone digestion and oil-gasoline extraction of insect fragments and rodent hairs in a separatory funnel. Their method is designed for spices, but some workers reported verbally that it had been used for flour. The method as adapted for flour in this laboratory is as follows.

To 50 g flour in a 1-liter Florence flask add 200 ml acetone in such a manner as to rinse down the neck of the flask. Immediately swirl until flour and solvent are mixed. Add 380 ml H_2O and 20 ml conc. HCl and mix. Add 20 ml light mineral oil. Place under reflux condenser and boil for 20 min. Transfer hot to a 2-liter separatory funnel rinsing flask with ca 200 ml of 1:1 H_2O-acetone. Allow to stand until mixture has stratified. Withdraw floury sediment and clear layer into another separatory funnel leaving the floating oily layer. Add ca 100 ml 1:1 H_2O-acetone to the first funnel and again withdraw floury material to second funnel leaving oily layer. Add 50 ml gasoline to the second funnel, mix vigorously, and allow to stratify. Withdraw and discard sediment from second funnel. Several times add ca 100 ml H_2O-acetone

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[3] *Methods of Analysis, A.O.A.C.*, 6th Ed., Sec. 42.28; *This Journal*, **29**, 63 (1946).

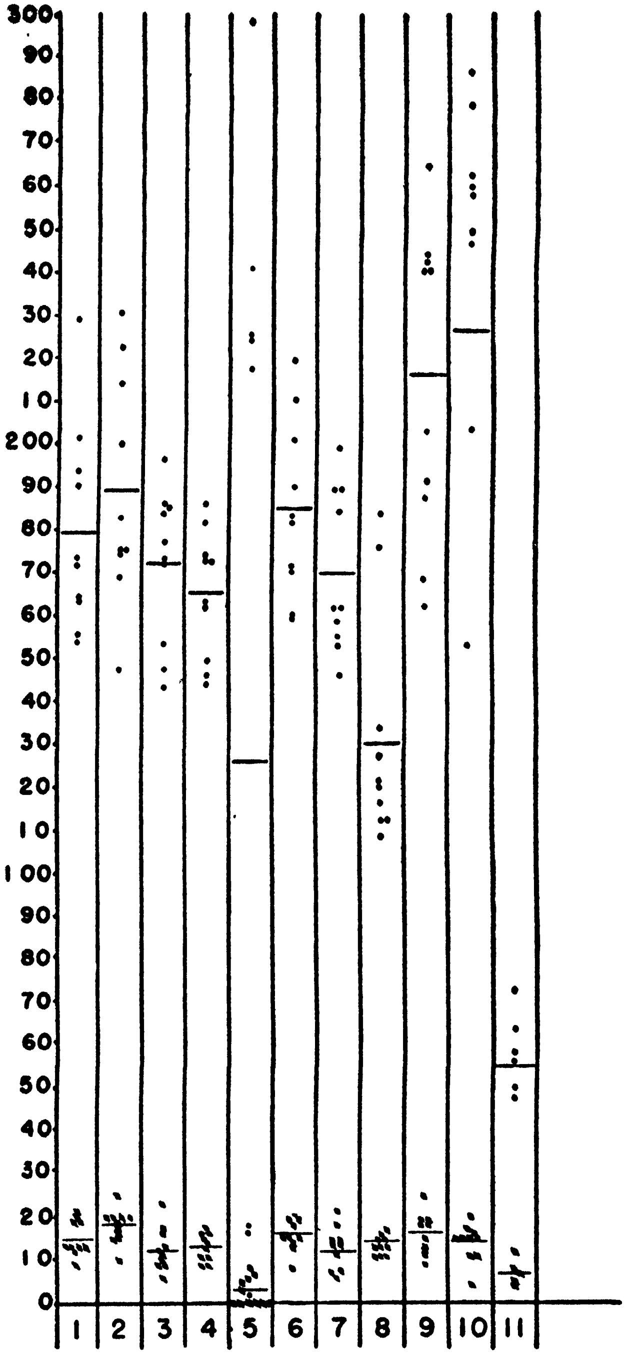

FIG. 1. Distribution of Insect Fragment and Rodent Hair Counts in the Eleven Series of Flours

- • = Insect fragment count
- ⸝ = Rodent hair count
- — Average, respectively, of insect fragment count (upper) and rodent hair count (lower).

and repeat, withdrawing and discarding sediment. Remove oil layer from both funnels to beaker, bring to boil in acid soln and filter.

The results obtained by this method are given as series 10 in Table 3. It is at once apparent that this series 10 procedure gave relatively high recoveries of insect fragments. The disconcerting point is that the method results in such a wide range of counts, subdivision 6 being markedly low, and the standard error of the average for insect fragments being 21.1. However, this procedure shows considerable promise. It specifically reopens the question of comparative recoveries using the Wildman trap flask (Howard, 1935) (6) as compared with a separatory funnel. The Wildman trap flask has been so successfully used to recover filth from such a wide variety of products that its inherent disadvantages often have been overlooked. Further work already initiated in this laboratory, evaluating the present recovery and loss of insect fragments and rodent hairs during the mechanics of trap flask operation, should illuminate this problem. In this regard it should be noted than when flour was digested in dilute hydrochloric acid, as described for series 9, a filth-bearing oily layer would not separate from the mixture in a separatory funnel with the same clear-cut division between oil and flour as was the case in a trap flask.

D. Use of sieves or silk bolting cloths

From time to time various workers in U. S. Food and Drug Administration laboratories have proposed the use of silk bolting cloths or metal sieves to retain insect fragments and rodent hairs and separate them from flour. Since the sieve sizes which they have suggested as being of possible value have approximately the same aperture sizes as the bolting cloths used in flour mills it is self-evident that extraneous material as well as the flour, will pass through.

Tests were made on five 50-gram portions of flour by the following method (Loughrey, 1945): (7)

Add 50 g of flour, with stirring to break up lumps, to soln of 30–35 ml HCl in 800 ml cold water in 1500 ml beaker. Bring to boil, stirring, and boil until thin and translucent. Filter thru #100 screen. Transfer material on the screen to ruled filter paper and examine.

Counts by this method (Series 11 in Table 3) very clearly show that insect fragments and rodent hair fragments in this flour are incompletely retained on a #100 seive, although a relatively higher proportion of the hair fragments are retained. Moreover, the filter papers obtained by this method contained more bran than when gasoline flotation was used, and the papers were more difficult to examine microscopically.

SUMMARY AND CONCLUSIONS

(1) Comparative data are given on the *A.O.A.C. Methods of Analysis*, **42.29** and **42.30** methods. Treatment of flour with chloroform as pre-

scribed by **42.29** markedly lowers the recovery of filth by **42.30**. Separate aliquots should be used for each section.

(2) There was no significant difference between the use of a 1-liter and a 2-liter trap flask, nor between pancreatin-digested and non-digested portions. Since the use of a 2-liter flask and the use of pancreatin results in the inclusion of less plant debris in the floating layer and less difficulty with routine samples, they should be used. Their use may be omitted when examining high patent flours.

(3) The use of water in the trapping procedure gave significantly higher recoveries than did the use of salt solution. The method should be so modified.

(4) The second "trapping off" recovers additional filth elements.

(5) The average insect fragment count using large quantities of dilute hydrochloric acid was as high as, or higher than, any of the averages for pancreatin digestion.

(6) Preliminary tests indicate that a new procedure, based upon the work of the American Association of Cereal Chemists, may give markedly improved recoveries. Further investigations are warranted.

REFERENCES

(1) Nicholson, J. F., and Walden, S., U. S. Food and Drug Administration Correspondence, March 15, 1944.

(2) Harris, K. L., and O'Brien, R. E., *This Journal*, **29**, 84 (1946).

(3) Eisenberg, W. V., U. S. Food and Drug Administration Correspondence on Spices (July 2, 1947).

(4) Amer. Assoc. Cereal Chem. Extraneous Materials in Flour (Mimeo.) (July 2, 1946).

(5) Walker, N. H. and Dalby, G., "Extraneous Materials in Ground Spices and Chocolate Products." *Cereal Chemistry*, **24**, 4 (1947).

(6) Howard, B. J., "Corn Worm in Tomato Products," *Food Ind.*, **7**, p. 321 (1935).

(7) Loughrey, J. H., U. S. Food and Drug Administration Correspondence (Jan. 1945).

TEST PAPER OF UREASE AND ACID-BASE INDICATOR FOR DETECTION OF UREA

By J. W. Cook (Food and Drug Administration, Federal Security Agency, San Francisco, Calif.)

The Marshall method (1) for urea makes use of urease to produce ammonia and carbon dioxide from urea. The increase in alkalinity of the solution is titrated with standard acid using an indicator which is not appreciably sensitive to carbonic acid. Urease papers, that is, filter paper impregnated with urease solution with subsequent drying, have been used as a convenient and stable laboratory preparation of the enzyme for a number of years (2) (3). Impregnating the urease papers with an appropriate indicator provides a convenient and delicate spot test reagent for urea in material such as urine-contaminated food products. In the absence of alkaline substances, the test is as specific for urea as is the enzyme employed. The absence of alkaline substances may be established through the use of an auxiliary test paper impregnated with the indicator alone. The test does not require the aid of the microscope as do the common methods for comparable amounts of urea (4). Agar plates with urease and indicator incorporated also give the same delicate test.

METHOD

PREPARATION OF TEST PAPERS

Urease soln.—0.2 g urease Squibb (or other suitable preparation) (2) (3) per 20 ml water. Make the dry powder into a paste by stirring with a drop or two of water and then dilute to 20 ml.

Brom thymol blue soln.—0.15 g brom thymol blue per 100 ml water. Make soln yellow with a few drops of 0.1 *N* HCl.

Preparation of paper.—Immerse pieces of heavy filter paper in the urease soln, remove, and allow the paper to dry partially. Then immerse in the indicator soln, remove, and hang the paper up until thoroly dry. The paper should be yellow. Preserve in a wide-mouth, dark bottle in a cool, dry place. The paper should be stable indefinitely. Prepare blank test papers similarly by impregnating with the indicator alone.

PREPARATION OF AGAR PLATES

Agar soln.—2 per cent. Bring 2 g agar plus 100 ml water to boiling, with stirring, and boil for a few minutes.

Brom thymol blue soln.—Same as for test paper.

Urease soln.—0.2 g per 10 ml. Prepare as for test paper except dilute to 10 ml.

Preparation of plate.—Melt some of the agar soln, filter thru two layers of cheesecloth, and allow to cool somewhat (but not sufficiently to set). Mix 10 ml of agar with 10 ml of urease soln, add 0.5 ml of indicator soln, stir well, pour into a petri dish, and allow to gel. The plates should be yellow. Prepare blank test plate similarly with indicator soln alone.

PROCEDURE

Place minute crystals of urea or urine-stained food material on both the agar-urease and agar-blank, or on moistened test paper and blank paper. (Urea produces a bright blue spot in 10–30 seconds which continues to increase in size for about 20 min. on the urease preparation, but does not produce color in the blank preparations. Alkaline salts produce color in both the urease and blank preparations. When alkaline salts are added to the moistened papers the blue color appears immediately after the alkaline salts are wetted, whereas urea crystals dissolve and seem to disappear; then after a period of 10 to 30 seconds the color appears. The color fades in about one hour.)

DISCUSSION

Agar plates afford a relatively large surface where a large number of determinations can be made at one time. The plate remains moist and is quite convenient for laboratory use. It has the disadvantage of instability of the enzyme, and may not be quite as sensitive as the test papers. The test papers have a great advantage in that the enzyme is in a dry state and therefore stable for long periods, unless exposed to adverse conditions of moisture, temperature, and light. The test paper can be carried by food inspectors for spot tests of food materials contaminated by rodent urine equally as well as it can be used in the laboratory.

ACKNOWLEDGMENT

The use of the urease-indicator test on paper was suggested by Doris H. Tilden.

REFERENCES

(1) MARSHALL, E. K., JR., *J. Biol. Chem.*, **14**, 283 (1914).
(2) KLEIN, BERNARD, *J. Lab. Clin. Med.*, **23**, 1097 (1938).
(3) HAWK, P. B., and BERGHEIM, O., "Practical Physiological Chemistry," Eleventh Ed., P. Blakiston's Son and Co., Inc. Philadelphia (1937), page 421, footnote 2.
(4) *Official and Tentative Methods of Analysis, A.O.A.C.*, Sixth ed., 1945.

CORRECTIONS FOR VOL. 31, NO. 3

In the report on Separation of Bromides, Chlorides, and Iodides in Drugs, by Newell E. Freeman and Beulah V. McMullen, the equation (4) on page 552, should read as follows:

(4) $X^- + 20 = X^+$

BOOK REVIEW

The Scientific Principles of Grain Storage. By J. A. Oxley, Northern Publishing Co., Liverpool, England. 96 pages plus references, index; price $1.72.

This book is largely concerned with heat, water, and air transfer in bulk stored grains. It is a phase of the grain storage problem in which Mr. Oxley is an authority and his treatment will be of help to persons engaged in research on this subject, since it points out the various factors concerned in grain storage. The factors themselves are treated with more words than substance.

K. L. HARRIS

NOTE

Note on the Determination of Phosphorus in Organic Compounds

By A. R. Wreath (Victor Chemical Works, Chicago, Ill.)

In the determination of phosphorus in organic compounds the semimicro Parr bomb method yields excellent results on all types of organic compounds, as was reported by Wagner *et al.* (1). This procedure is considerably shorter than that recommended by Hardin and MacIntire (2). We use this combustion method on samples of 50–80 mg and follow the A.O.A.C. volumetric procedure (3) after the excess sodium peroxide has been destroyed and the soln neutralized with HNO_3.

We also use an acid digestion on some samples after first checking the results against those obtained by the Parr bomb combustion. This procedure requires less apparatus and permits the handling of a larger weight of sample. The method is as follows:

Weigh accurately ca 0.5000–1.0000 g sample into a 400 ml beaker. Add 50–60 ml HNO_3 (Sp. gr. 1.42) and boil down to ca 25 ml. Add 15–20 ml $HClO_4$ (60–70%) and evaporate to dense fumes of $HClO_4$ and a colorless soln (Note 1). Cool. Dilute to 250 ml volume, neutralize a suitable sized aliquot, and complete the determination by the A.O.A.C. volumetric procedure (3).

Note 1. If the soln turns brown while being evaporated to fumes, remove from hot plate at once and add a few ml of HNO_3. Replace on hot plate and continue. This may have to be repeated more than once. An explosion may result if this is not done.

Both procedures yield excellent results. No trouble has been experienced with the HNO_3-$HClO_4$ digestion. Note 1 was added to the procedure as a precautionary measure. The preliminary digestion with HNO_3 serves to reduce the organic material to a safe level prior to the addition of $HClO_4$.

Some typical results:

	P_2O_5 CONTENT	
	HNO_3-$HClO_4$	PARR BOMB
	per cent	*per cent*
Hexaethyl tetraphosphate[a]	57.6	57.8
Tetraethyl pyrophosphate	49.0	49.0
Tributyl phosphate	33.8	34.0
Tricresyl phosphate[b]	19.0	19.3
Triphenyl phosphate[b]	22.0	22.1

[a] Commercial preparation.
[b] Practical grade.

REFERENCES

(1) Wagner, *et al.*, *Ind. Eng. Chem., Anal. Ed.*, 15, 149–151, and 601–602 (1943).
(2) Hardin and MacIntyre, *This Journal*, 31, 400 (1948).
(3) *Official and Tentative Methods of Analysis*, Sixth Ed., p. 22, 1945.

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